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Bottom-up effects of eutrophication on insect interaction networks

BOTTOM-UP EFFECTS OF EUTROPHICATION ON INSECT INTERACTION NETWORKS

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ABSTRACT

The need to increase productivity in grasslands has led to the addition of nutrients to the point where plant species have been lost. The cascading effect of this loss on the community of associated species is not well understood. The aim of my thesis is to study how variation in soil nutrients – both natural and by the addition of fertilisers - changes plant community composition and how these changes affect the structure of plant-flower visitor networks, plant-herbivore networks and herbivore-parasitoid networks.

There are three parts to my study. First, I tested if high fertility decreases insect specialization using natural variation in soil fertility and a nutrient enrichment simulation. I observed that higher nutrients led to less specialist, but more robust ecological networks in both current and simulated scenarios.

In the second study, I evaluated the effects of a 27-year experiment, which added both organic and inorganic fertiliser to replicate plots, on the three types of network. Eutrophication decreased the number of forb species, and this changed the structure of the ecological networks. These changes however, took place with no change in insect species richness.

Finally, I compared the performance of organic and inorganic fertilisers on the production of livestock forage alongside its impact on biodiversity, asking if there is an optimum fertilizer strategy where both livestock and biodiversity can co-exist? While there was no fertiliser treatment that had the best performance for all the variables studied, the application of farmyard manure offered the closest to a win-win scenario.

Looking forward, to evaluate the consequences of eutrophication and achieve sustainable production in grasslands, an interdisciplinary approach is needed where ecologists and livestock managers work closely together to identify management solutions acceptable to both.

DEDICATION

To all my loved ones and you

Si se puede

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration, or the assistance of, others is indicated as such. Any views expressed in the thesis are those of the author.

Edith Villa Galaviz

Bristol, September 2018

“Think left and think right and think low and think high.
Oh, the thinks you can think up if only you try.”

Dr. Seuss

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

"Understanding the rules responsible for who interacts with whom,
as well as the functional consequences of these interspecific interactions
is central to predict community dynamics and stability"
Bartomeus et al. *2016*

Eutrophication or nutrient enrichment is a process during which the amount of nutrients in the system increases to levels where species extinction occurs (Bouwman et al. 2002). Better known for its impacts on aquatic systems, eutrophication also affects terrestrial habitats such as moorland, heathland and grassland (Stevens et al. 2010a, Payne et al. 2013), where it is regarded as one of the most important causes of biodiversity loss in this century (Tilman et al. 2001, Maskell et al. 2010, Hautier et al. 2015). In these cases, the loss of plant species is not mainly due to toxicity, but rather to changes in the competitive hierarchy among plant species (Dickson and Foster 2011, Farrer and Suding 2016). Species that can capitalize high inputs of nutrients grow faster and outcompete others by impeding access to light, thereby reducing survival of their seedlings (Hautier et al. 2009, DeMalach et al. 2017) and later, reducing their seedbank (Basto et al. 2015).

While evidence of the effects of nutrient enrichment in soil and plant communities is widespread, studies on the impacts on primary and secondary consumers communities is "patchy at best" (Stevens et al. 2018). Changes in plant communities are expected to influence plant availability and plant quality for primary consumers with potentially knock on effects for the secondary consumers (Lavorel et al. 2013, Nijssen et al. 2017). However, the influence of nutrient addition on the interactions between plants and their invertebrate secondary consumers is still not well understood (Humbert et al. 2016), this being particularly true in agroecosystems, one of the most affected habitats (Habel et al. 2013).

In this thesis, I study how soil nutrients, through the modification of the plant community, affects both the plant-flower visitor network and the plant-herbivore-parasitoid network (network referring to the networks of interactions between species). I do this by using grasslands as my study system, along with a natural soil fertility gradient and a long-term fertilisation experiment. In this introductory chapter, I will first explain the conceptual model of the thesis. Then, I provide the background for my research on which the conceptual model is based where I explain the effects of nutrient enrichment on plant and insect communities in grasslands which is my study habitat. Finally, I outline the aims of my study and the thesis structure.

In the following paragraphs and chapters, I will use different concept which I define as follows:

Soil fertility: chemical (nutrients levels and pH) and physical (moisture) properties under each of the plant communities grow.

Palatability: characteristics than make a leaf edible for an herbivore (i.e texture and anti-herbivore defences).

Fllower visitors: individuals of species that visit a flower but not necessarily pollinate the flower while pollinator will mean that the animal species deposits a viable pollen grain that will developed into a fruit. This means that while all pollinators are flower visitors not all flower visitors are pollinators. Because, I did not test any seed production or pollen deposition, I decided to use the word flower visitor when talking about the insects recorded in each of the studies.

Herbivore: although this concept includes all animals that feed on any part of a plant (i.e. flowers, leaves, roots), I used the word to refer to immature states of folivorous insects (that feed on leaves). It is not uncommon in herbivory research to use the word herbivore when referring to a single guild of herbivores (see Carvalheiro et al 2008, Villa-Galaviz et al. 2012).

Network specialization: degree in which species of one trophic level interact (i.e. plants) or not with most or all species of the other trophic level (i.e. flower visitors) within the network.

Network robustness: capacity of the network to keep their characteristics (i.e network specialization, species richness) after species are removed from the networks.

CONCEPTUAL MODEL

As illustrated in figure 1.1., nutrient inputs from the atmosphere and human actions such as use of fertilisers needed to increase food production, releases belowground competition for plants while increases the aboveground competition. Both ecological processes have two main consequences on the plant communities: a) loss of species that later leads to a replacement of forbs by grasses and b) changes in the nutritional content of plants. Such changes affect different species composition and species traits (i.e. body size) of insect communities from pollinators, herbivore to parasitioids. Such changes affect the structure of the ecological networks by modifying the manner species interact with each other. Insect species could change their interacting species by: a) changing interacting partners known as "rewiring" of interactions, for example, feeding on a species that is more abundant or more attractive (i.e. plants with higher palatability or preys of higher body mass; and b) declining species richness. Loss of species means a loss of resources for insects which consequently can provoke: i) dominance of generalist species as the species more likely to cope with the loss of resources which as consequences could diminish the network specialization; and ii) more species of the second trophic level per species of the first trophic level (known as increase in network asymmetry) caused by a decline the loss of species.

In this thesis, I focused on the route of the cascading effects of loss of species from plants to parasitoids communities. I tested its effect on network specialization and asymmetry of the network (measured as vulnerability and generality in chapter 3). I also cover the need of the addition of nutrients in grasslands in the form of addition of fertilisers which is the question address in chapter 5 where I tried to answer if there is a sustainable fertiliser practice for the conservation of insect communities.

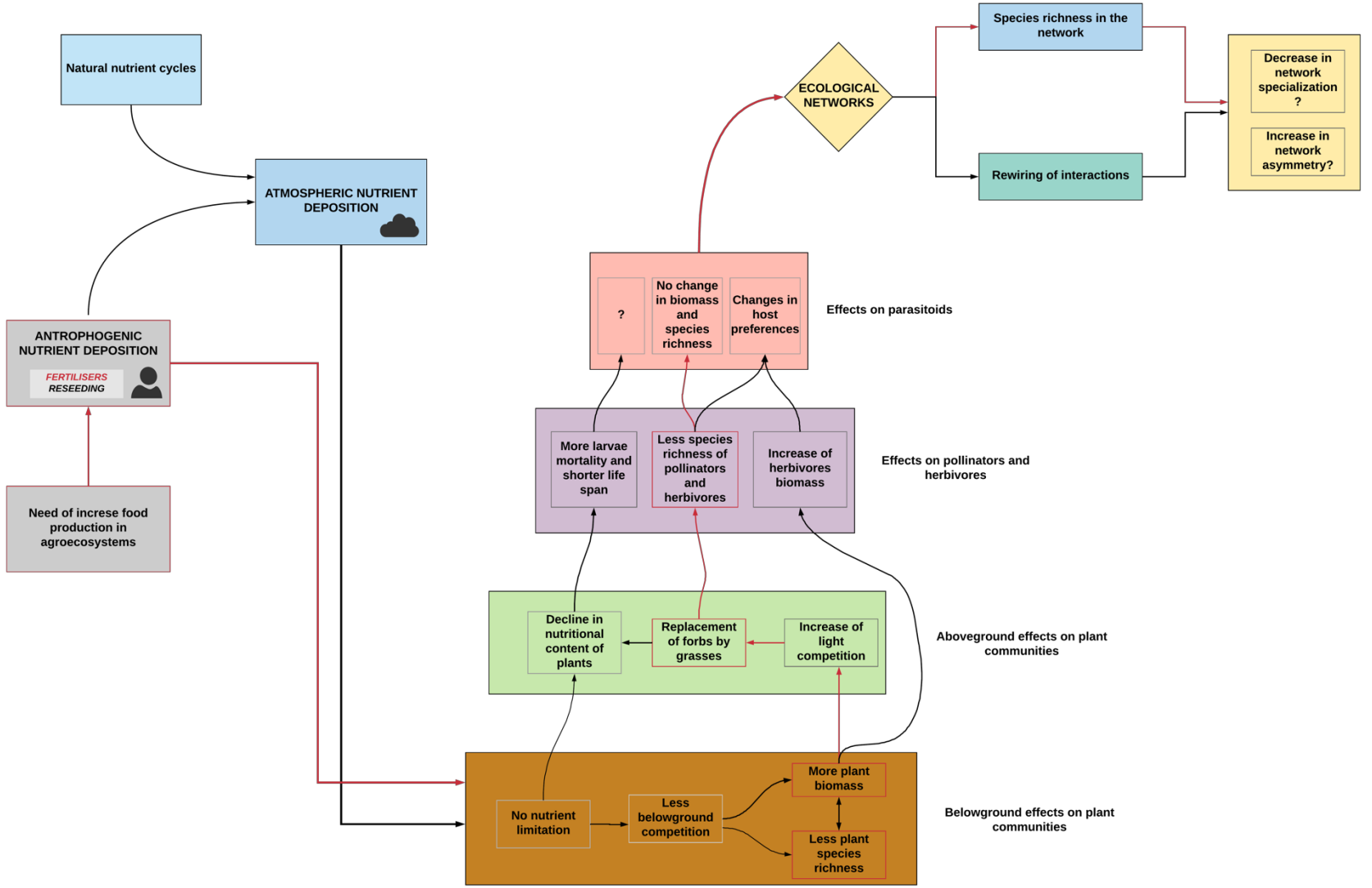


Figure 1.1 Conceptual model of the thesis. Sources of nutrient enrichment and consequences in each of the trophic levels studied, represented in the pyramid at the centre are represented in the figure, as well as the expected consequences in the structure of the ecological networks. The fundaments of the thesis as well as the general question that it covers are the ones indicated in the red route.

BACKGROUND

Current approaches to the study of eutrophication

Eutrophication can be triggered by the increase of a variety of nutrients such as: phosphorous (P), sulphur (S), carbon (C) Potassium (K) and Nitrogen (N), either individually or in combination (Fay et al. 2015). Nitrogen however has received by far the most attention to date. Studies have shown that many human activities such as fuel combustion in industry, the use of nitrogen fertilisers, nitrogen fixation harnessed for agricultural purposes and other human activities have approximately doubled the emission of N worldwide (Millenium Ecosystem Assessment 2005). This increase in nitrogen is a cause of great concern as multiple habitats have been impoverished in diversity in many countries. Even in the most optimistic future scenarios nitrogen inputs will still exceed sustainable boundaries (Winiwarter et al. 2013).

Nitrogen enrichment increases plant primary production and at the same time it reduces plant species richness (Foster and Gross 1998, Stevens et al. 2004, Dupré et al. 2010, Maskell et al. 2010, Stevens et al. 2010a, Bassin et al. 2013, Roth et al. 2013). This extinction occurs at, or below, the range of the nitrogen critical load (10-15 kg N ha^{-1} y^{-1}; Payne et al. 2013) and it has an cumulative effect (De Schrijver et al. 2011). An increase in nitrogen affects plant communities via four main mechanisms (Phoenix et al. 2012): 1) a decline in biodiversity via the expansion of nitrophilous species and competitive exclusion of others; 2) toxicity; 3) soil acidification, and 4) Increase in the susceptibility of plants to secondary stresses (e.g. herbivory). In Great Britain, changes in plant species composition associated with Nitrogen deposition have been reported at a countrywide scale (Smart et al. 2003, Stevens et al. 2004). Most plants in the UK have shown a decrease in distribution or disappeared in some localities (Smart et al. 2005), with few species becoming more abundant (Smart et al. 2000, Smart et al. 2003, Stevens et al. 2004, Cleland and Harpole 2010).

Given the diversity loss by nitrogen deposition observed in the last decades, the reported effects on plant and insect communities have been reviewed by several authors. I summarize the main conclusions of these reviews in the following paragraphs with emphasis on the findings for insect communities:

Throop & Lerdau (2004) emphasize the changes in host quality which they believe release some insect populations from nutrient limitation and anti-herbivore defences, these having the potential to disrupt the ecosystem functioning if populations are not controlled by natural enemies. Tylianakis et al. 2008 comment on how nitrogen deposition together with other global

drivers of species loss have altered competitive interactions among plant and animals and how they can weaken mutualisms such as pollination, enhance herbivory and have variable effects on predation. Lavorel et al. (2013) identify the response traits of the species to eutrophication and acidification as the main gap in knowledge. They proposed a theoretical model that links plant responses and effect traits (i.e. specific leaf area, plant morphological type, flower morphology) to pollinators and herbivores response traits (i.e. body size, proboscis length, feeding guild) showing that a modification of species composition of the whole system is expected under eutrophication.

In a more recent review, Nijssen et al. (2017) identify 5 main pathways through which nitrogen deposition affects fauna in terrestrial systems: 1) a levelled and humid microclimate by favouring tall species plants, 2) decrease in reproductive habitat, 3) changes in food plant quantity, 4) changes in nutritional quality of food plants and 5) changes in the availability of prey or host species due to cumulative effects in the food web. Finally, Stevens et al. 2018 report that even though there are a low number of studies on primary consumers, these provide consistent evidence of negative effects on both pollinators and folivorous insects. Overall, Bobbink et al. 2010 found a large body of evidence that nitrogen accumulation is the main driver of changes in species composition across different ecosystems around the world.

Regardless of the focus on nitrogen enrichment, enrichment by other or multiple nutrients appears to be equally important, though much less studied. A review by Peñuelas et al. 2013, shows that anthropogenic inputs of carbon, nitrogen and phosphorus into the biosphere are affecting the life histories and competitiveness of microbes, plants and animals species. Authors like Fay et al. 2015 believe that multiple-nutrient limitations must be considered when assessing the ecosystem-scale consequences of nutrient enrichment. Indeed, nutrient enrichment by phosphorous is known to have a large impact on biodiversity in grasslands (Ceulemans et al. 2011), sometimes higher than nitrogen (Ceulemans et al. 2013) and with stronger effects on insect communities (Vogels et al. 2017). Harpole & Tilman 2007 observed a decrease in plant species richness when increasing numbers of limiting soil resources were added (soil moisture, nitrogen, phosphorous and base cations). However, they found that only plots which received all the soil resources were significantly more productive and significantly less species rich. Similarly, Harpole et al. 2016 added different nutrients both individually and in combination and observed that plots receiving a single resource treatment (N, P and K) diverged as much from each other as they did on average from the control plots, with the higher negative effects when applied in combination.

Although, much less studied, the changes in plant community due to nutrient enrichment have been observed to indirectly affect arthropod community composition by changing the abundance of some guilds after both short and long-term fertilisation (Butler et al. 2012, Evans and Sanderson 2018). In this sense, increase of nutrients in isolation (e.g. atmospheric deposition of nitrogen) or in combination (e.g. addition of fertilisers) is an important human disturbance of arthropod communities. Moreover, we have a poor understanding of the underlying mechanisms causing these changes, which is of concern for habitats under constant manipulation of nutrients such as grasslands (Stoate et al. 2009, Habel et al. 2013).

Grasslands cover approximately of 20-40 % of the surface of the planet (FAO 2015a) and approximately 44% of the UK (Office for National Statistics 2015). They are semi-natural habitats in which domestic animals are an integral component (Poschlod and WallisDeVries 2002). When not managed for agriculture, grasslands are hot spot of biodiversity (Habel et al. 2013). Alteration of nutrient inputs is part of both traditional production management and the conservation management of grasslands. This alteration of nutrient inputs happens by removing nutrients through grazing and mowing; and increasing nutrients directly both by applying fertilisers and indirectly by reseeding (adding seed changes the physical properties of the soil making nutrients more available for plant uptake (i.e. Bhogal et al. 2000). Unfortunately, increasing grassland productivity for farming requires the addition of fertilisers which has the side effect of reducing its diversity. It is estimated that around 50% of English grasslands has been transformed to agriculturally improved grasslands over the last 32 years (Ridding et al. 2015). Fertilisation by organic and inorganic fertilisers, along with atmospheric nitrogen deposition, have made grasslands one of the habitats most threatened by eutrophication (Habel et al. 2013).

Although, nitrogen emissions and use of fertilisers have dropped slightly in the UK over the last 30 years (DEFRA 2018a) it remains a challenge to maintain productivity while not detrimentally affecting native species. It's a challenge because the amount of fertiliser applied in grasslands to improve productivity under protection schemes (25 kg ha^{-1}) is sufficient to cause species extinctions (Smith et al. 2008b). Ironically, fertilisers can be needed to restore botanic biodiversity in degraded grasslands (Smith et al. 2008b) where they increase the abundance of some invertebrate species (Vickery et al. 2001). Understanding how eutrophication affects insect communities to maintain livestock productivity without adverse effects on the plants and insects they support, is important to secure the ecosystem services that this habitat provides.

Effects of eutrophication from soil to plant communities

As mention before increase of nutrients leads to a decline in plant species in which forb specie are the most affected group while most grasses increase their biomass (Isbell et al. 2013). Soil as the place where plant take nutrients, represent a start point of this succession process. However, the mechanisms that triggers these non-random extinction events are rather complex, since responses depend on different aspects of the physiology of the plant species or the chemical form which nutrients are released into the soil. For example, inputs of atmospheric nitrogen in form of NH_4^+ in places where NO_3^- is the dominant nitrogen form can have toxic effects on sensitive species. It can also cause soil acidification and enhance availability of metals like Al^{3+} (aluminium) or Fe^{3+} (Iron) which are toxic for some species, where the magnitude of such effects will depend on the pH in soil, previous nutrient content in soil and diversity of the habitat (Phoenix et al. 2012). On the other hand, phosphorous unlike nitrogen or carbon does not have a stable gaseous phase in the Earth's atmosphere. Instead it mainly proceeds from rocks in compounds with other nutrients such as Al or Fe (Mahowald et al. 2008). Being part of such compounds made phosphorous hard to be released for plant uptake having a strong cumulative effect. However, the mechanism trough which phosphorous affects plant communities through soil is still not well understand. In addition of this individual effects, the effect of nutrient addition tend to be additive, for example, it was found in a meta-analysis that in 28% of studies community biomass responded only with addition of both nitrogen and phosphorous (Harpole et al. 2011). For this reason, it is hard to established a simple explanation of the synergetic effects of the increase of different nutrients from soil to plant communities.

In this sense, a model based on the ecological process that occur belowground and aboveground as the one proposed by Harpole et al, 2016 offer the best approach to understand the synergetic effects of eutrophication that leads to a decline of biodiversity in grasslands. The model of Harpole refers to the process that occur in soil that beneficiate some species and negatively affect others, as the loss of ecological niches for plants given the loss of competition for soil resources (belowground) which provokes an increase in the competition for light (aboveground; fig. 1.1.).

The model considers the fact that in grasslands, nutrients such as phosphorus and nitrogen exist in low levels. Such limitation has created a variety of ecological niches or different adaptations in plant species to cope with these limitations, such as association with bacteria (i.e. legumes) and fungus (mychorrizas), growing rates etc. When nutrients are added, plants are released from competition for nutrients making some strategies no longer an advantage (i.e. association with N fixing bacteria). In turn, species that can make use of nutrients quicker, increase their leaf biomass

and become better competitors aboveground (mainly grasses and few forbs species). These species, then cover the light for other species that cannot grow as fast or taller (most forbs species). This limitation in light affects the photosynthesis of these short species altering their seed production finally causing their extinction.

The effect of nutrient enrichment from insect individuals to insect communities

Effects of the increase of nutrients, particularly of nitrogen which is the nutrient that has been most widely studied, go from modification of species functional traits to changes in the structure of the plant and insect communities. In the following paragraphs, I provide a summary of the main findings on changes in insect traits and communities. Studies at community level remain very rare, so in this section I highlight on findings at the species level as an introduction for "the ecological network approach" which is the main focus of the thesis.

Plant-pollinator networks

Several plant traits important to pollinators such flower abundance, nectar composition (sugar composition, and sugar and amino acid concentration) and bloom duration vary according to nutrient enrichment. Under high levels of Nitrogen, at the species level, flower size and abundance increase (Burkle and Irwin 2010, Hoover et al. 2012) while the amino acid composition of the nectar and pollen changes (Gardener and Gillman 2001, Ceulemans et al. 2017). Those changes make species more attractive for flower visitors so they receive more visits (Burkle and Irwin 2010, Hoover et al. 2012) and thereby produce more seeds (Muñoz et al. 2005). Nevertheless, these apparently positive effects can be offset by negative effects. Hoover et al. (2012) observed that pumpkin plants fertilized with nitrogen attracted individuals of *Bombus terrestris* L. which consumed more nectar, but due to the decrease in phenylalanine concentration and higher sucrose to hexose (fructose and glucose), this preference reduced the survival of bumble bees by 22%. They also observed a shorter bloom duration, which reduced the life of the pollinators due to a mismatch in the interaction; a similar effect was reported by Ceulemans et al. 2017 in a fertiliser addition experiment. Authors observed the larval mortality of *Bombus terrestris* L. feeding on *Succisa pratensis* Moench increased due to a decline of essential amino acids in the nectar and pollen; and decreased in fructose content in pollen. Other experiments have shown that plants under fertilizer addition has pollen of higher diameter but with lower pollen production (Muñoz et al. 2005, Wu et al. 2010). The implication of such changes in diameter are still unclear but these changes could diminish the fitness of the plants.

Life history traits are important to determine the reproductive response to soil nutrient. In an resource addition experiment involving the monocarp *Imopsis aggregata* and the perennial *Linum lewisii*, after one year of fertilization the former increased flower production, bloom duration, corolla width, nectar production, aboveground biomass, and pollen; while the latter showed no change in growth or reproductive output with exception of a lengthened bloom duration (Burkle and Irwin 2008). So, at higher nutrient availability some species could increase their fitness (Muñoz et al. 2005) whereas others do not (Burkle and Irwin 2008), resulting in an uneven distribution of pollinators over the available floral resources as reported by Burkle and Irwin (2010) .

Plant-Herbivore-Parasitoid interactions.

Changes in plant chemistry due to nutrient enrichment are an important driver of species loss for invertebrates (i.e Vogels et al. 2017) . Nitrogen enrichment releases herbivores from nutrient limitation (Mattson 1980) and carbon based defensive compounds (Throop and Lerdau 2004). A reduction in carbon defensive compounds along with higher nitrogen concentrations from the addition nitrogen based fertilisers makes leaves more palatable, boosting the abundance and biomass of herbivores (Moon and Stiling 2000, Stiling and Moon 2005, Eatough Jones et al. 2008, Butler et al. 2012, de Sassi and Tylianakis 2012). However, addition of both nitrogen and phosphorous can also change the ratio of nutrients such as C:N and N:P, where for example an increase of nitrogen could reduce the soil phosphorous availability or promote other anti-herbivore defences, negatively affecting species unable to cope with nutrient limitation or new anti-herbivore defences (Vogels et al. 2017)

The response of parasitoids to change in herbivores varies (Tylianakis and Binzer 2014). An increase in herbivores biomass, increases parasitoid biomass but with no overall effect on parasitism rate at community level (de Sassi and Tylianakis 2012). de Sassi et al. 2012b, observed that parasitoids respond to the increase of biomass of herbivores by a preferring bigger herbivores, but with no effect on parasitism, probably because the alpha diversity of herbivores was unaltered by nitrogen addition. The amount of research at the single species level is large (Tylianakis and Binzer 2014, La Pierre and Hanley 2015), however given that responses to nitrogen and nutrient enrichment are interspecific it is hard to extrapolate such responses at community level, and more importantly, hard to detect possible consequences in the functionality of the system. This highlights the importance of using tools that allow the evaluation of responses of the different species and guilds present in a habitat simultaneously.

Ecological networks as a tool for understanding the bottom-up effects of eutrophication

Because the consequences of perturbation on ecosystems services can be easily overlooked when focusing on single trophic level or guild (Tylianakis et al. 2007, Tylianakis et al. 2010), it is now accepted that the interactions between species need to be considered in addition to diversity or species richness. Ecological networks are descriptors of ecological systems which consider both the species and the interactions between species (Bascompte 2009). They are helpful tools when assessing human impacts on natural communities (Memmott 2009) and ecosystem services (Montoya et al. 2003, Tylianakis et al. 2010).

Ecological networks are built by recording the frequency of interaction between individuals from different trophic levels. Networks provide information on the generalization and specialization of species (Lewinsohn et al. 2006), measured by counting the number of interactions a species establishes with other species in the network. The number of interactions determines if a species is a core generalist species or a specialist peripheral species within the network (Dáttilo et al. 2013).

Most of the consequences of eutrophication reported in the literature are from nitrogen deposition studies or networks of a single plant species or a handful of species from the first and second trophic levels (Moon and Stiling 2000, Stiling and Moon 2005). This isn't ideal given that the expectation is that the effects of eutrophication could potentially propagate through the food web from plants to parasitoids (Natalia Lescano et al. 2012, Erisman et al. 2013). While evidence at community level is scarce, what data there is shows a consistent decline in both host availability and quality of resources for insects (Lavorel et al. 2013, Nijssen et al. 2017). For example, a decline in floral abundance, caused by the decrease of forbs and dominance of grasses following nitrogen deposition (Stevens et al. 2006, Phoenix et al. 2012), is believed to underlie the loss of some key plants which provide resources for long-tongued bees (Stevens et al. 2018). For herbivore and parasitoids communities, Sassi and collaborators (2012a) observed that under elevated nitrogen, a different increase in biomass in herbivore and parasitoids where herbivores doubled their biomass while parasitoids have no significant increase. Other studies have found changes in distribution of herbivores, where species that feed on nitrophilous and polyphagous species increase at the expense of non-nitrophilous species (Smart et al. 2000, Öckinger et al. 2006, Pöyry et al. 2017). However, many of the potential changes in food webs remain unknown, for example whether food webs become more generalist or interconnected given the loss of specialist species?

Systems with high species richness tend to be more complex in terms of species arrangement within the ecological network (e.g. Montoya et al. 2003). This complexity provides

resilience to the network as more functional redundancy is expected (Memmott et al. 2004). Because eutrophication decreases species richness, food web complexity could be affected. By reducing the complexity of the network, eutrophication could also affect other web properties related to distribution of interactions within species other web metrics, for example the stability of the network which depends on the species richness (Dunne et al. 2002, Dunne and Williams 2009). A reduction in plant richness results in less diverse herbivore (Cleland and Harpole 2010) and pollinator community (Carvell et al. 2006, Hudewenz et al. 2012, Wesche et al. 2012). Loss of species richness, result in networks with higher connectance, less interaction diversity and lower mean interaction strength (Rzanny and Voigt 2012) as well as shifts in the relative dominance of coexisting plant and animal taxa, and increase or decrease of predation (Tylianakis et al. 2008). Such simplification of the network not only reduces the tolerance to disturbance of the network because of lower functional redundancy (Memmott et al. 2004, Bascompte 2009) but also could affect the efficiency of ecosystem services (Montoya et al. 2003).

To my knowledge just three studies have used an ecological network approach for studying the effect of eutrophication. Burkley & Irwin 2009 analysed the plant-pollinator network after three years of nitrogen addition and found that even though pollinators visited different numbers and type of plant, the nested pattern and the frequency of interaction of the network remained unchanged . Fonseca et al. (2005) found a strong effect of soil fertility in abundance and richness of plants and insect herbivores but a weak effect on the connectance of the webs in a natural soil fertility and altitudinal gradient. The third study is a theoretical evaluation of the potential impact of eutrophication on a size-structured parasitoid-host networks, for which Binzer et al. 2016 found that the destabilizing effect of eutrophication on the structure of the network was higher than the destabilizing effect of diversity loss.

AIMS OF THIS STUDY

The overall aim of this study is to determine how soil nutrients affect both insect food webs and pollination networks in grassland, these effects being mediated via changes in plant community composition. My thesis is made up of five chapters overall: this introductory chapter, three data chapters and a general discussion chapter. The first two data chapters are based on field studies on the effect of nutrients on ecological networks, while the third chapter uses data on plant productivity and nutrient content, along with the data collected in the previous chapter, to explore whether sustainable livestock production is a realistic proposition? Thus, can we can have both animal production and biodiversity in grasslands?

In *chapter one*, I review current approaches to the study of eutrophication and their limitations, I introduce grasslands as the focal habitat and discuss the effect of nutrient enrichment on species and communities of interacting species.

In *chapter two,* I undertake a study in the urban grasslands in Bristol in which, I correlate soil fertility measures and specialization of the plant-insect network. Specifically, I test if the tendency of generalist species to increase following nitrogen deposition in a natural soil fertility gradient and the robustness of the plant-pollinator and plant-herbivore-parasitoid networks. I also simulate the effect of nutrient enrichment on the interaction network structure under a low eutrophication scenario. This work was conducted during the first year of my thesis and is based on data from 255 species interacting in 12 plots.

In *chapter three*, I evaluate the impact of 27 years of experimentally manipulating the fertiliser addition of a hay meadow on plant-pollinator networks, plant-herbivore networks and herbivore-parasitoid networks. I test if changes in plant community composition (decline of forbs species and dominant plant species), affects the structure of the insect network, focusing on the effects on the vulnerability and generality of the network. This work was conducted during the second and third years of my thesis and is based on data from 191 species interacting in 72 plots.

In *Chapter four* I ask whether there is a fertiliser management practice that can maintain livestock production whilst not negatively affecting insects in temperate grasslands. To do this, I use information on plant production from previous studies at the field site, my data from chapter three, and complementary data I collected on the nutrient content of the vegetation. This work was conducted during the third and fourth years of my PhD.

Finally, in *Chapter five*, I discuss the main contributions and limitations of the thesis to the knowledge of the bottom-up effects of eutrophication on insect food webs and suggest future directions.

CHAPTER TWO

THE EFFECT OF SOIL FERTILITY ON INSECT INTERACTION NETWORKS

Authors' contributions

Jane Memmott and Edith Villa Galaviz conceived the main idea. Edith Villa Galaviz conducted the sampling and analysis. Simon Smart and Jane Memmott helped with field logistics and analysis. Elizabeth Clair and Edith Villa Galaviz conducted the bioinformatic analysis. Ian Vaugham assisted with statistical analyisis.

ABSTRACT

Nutrient enrichment is one of the greatest concern of biodiversity conservation of this century. Studies have reported a decline of species richness together with a change in the distribution of communities of Lepidoptera mainly related to the loss of plant species caused by nutrient enrichment. These studies also showed that these changes have beneficiated mainly generalist species and few specialist. However, any of these studies have evaluated if this plant mediated effects, affect other insect communities. Particularly if nutrient enrichment also alters the specialization of the ecological networks. They also lack to explain how natural gradient of nutrients affects the specialization of the network and the future tendency if nutrient enrichment continues. In this chapter, I tested if places with higher levels of nutrients tend to have less specialist ecological networks and how nutrients affect the robustness of the network. I also tested, if such tendency would continue under a hypothetical nutrient enrichment scenario. To achieve it, I studied the specialization and robustness of 12 plant-flower visitor networks and 12 plant-herbivore-parasitoid networks from plots with different levels of nutrient (i.e. %phosphorous and % nitrogen). Based on the habitat preferences of the different plant species to soil fertility and pH, I also simulated the effects on each network that future extinctions due to nutrient enrichment could occur. Then, I compared the network specialization and robustness of the networks before and after the species extinctions. I found that higher levels of different nutrients are related to lower values of network specialization but higher robustness given the higher interconnectivity between species. Simulated networks showed lower levels of network specialization than the original networks regardless of the previous soil fertility of the habitat. It also remarks the fact that keeping emphasis on a single nutrient neglects the fact that in natural conditions, different nutrients play an interactive role in structuring the interactions within the network.

INTRODUCTION

Along with climate change and land transformation, nitrogen deposition is one of the greatest threats to biodiversity in the 21th century (WallisDeVries and Bobbink 2017). Increase in nitrogen causes competition among plants that ultimately leads to functionally impoverished habitats (Hautier et al. 2009, Phoenix et al. 2012, Basto et al. 2015). Although, nitrogen deposition is the most studied nutrient (Ceulemans et al. 2011, Ceulemans et al. 2013), elements such as phosphorous, carbon and potassium have also been identified as global drivers of vegetation changes and plant species loss (Harpole et al. 2016, Soons et al. 2017). In fact, the effect of enrichment by multiple macronutrients is usually more potent than that resulting from luxury levels of a single nutrient (Harpole and Tilman 2007, Harpole et al. 2016) not only in structuring plant communities (Crawley et al. 2005) but also invertebrate communities (Evans and Sanderson 2018).

Research on the effects of nitrogen enrichment on insect communities has shown that in addition to species loss (Öckinger et al. 2006) there can be an increase in insect biomass due to a higher host palatability (Throop and Lerdau 2004, Stiling and Moon 2005) and alterations of pollinator floral preferences (Burkle and Irwin 2008, Hoover et al. 2012). Nitrogen enrichment can also change the degree of specialization in insect communities (Pöyry et al. 2017, Chapter 3). Studies on the distribution of European Lepidoptera species have shown a historical tendency for polyphagous or generalist species to increase, along with species whose larvae specialize on nitrophilous host plants (Smart et al. 2000, Pöyry et al. 2017). These studies, however, are based on data from the literature or survey data, rather than experimentally induced interactions in the field, and so provide limited detail on the dynamic responses of varying trophic levels to changing soil fertility.

Another way to measure the diet breadth, and therefore to unpick the responses of specialists versus generalists, is to use an ecological network approach as these provide detailed information on observed interactions between species in the field. They better evaluate potential impacts on ecosystem services and may be more accurate than correlational changes in the geographical distribution of species. Rather few studies use this approach, one that does, Burkle and Irwin (2009), varied nitrogen inputs into experimental plots and evaluated the effects of plant-pollination networks. They did not observe changes in the generalisation of the species in the ensuing networks, but they only identified species to the family level, which makes it difficult to accurately analyse network structure (Lewinsohn et al. 2006, Jordano 2016) and so their conclusion needs to be treated with caution. A second study was done using a natural soil fertility

gradient in a shrublands-woodland gradient in Brazil. Here, Fonseca et al. 2005 observed that changes in soil fertility affected the abundance and richness of plants. The authors found that increasing species richness diminishes the connectance of the plant-herbivore-parasitoid network. However, this result was not explained by soil fertility but by changes in species richness. Currently, how soil fertility affects the interactions between species, and specifically how it affects their diet breadth remains unknown.

In this first chapter, I used a correlational approach to investigate how soil fertility affects the interactions between species. First, I focus on the relationship between soil fertility and two network variables - network specialization and network robustness - as structural summaries of the architecture of three types of insect ecological networks (plant-pollinator, plant-herbivore and herbivore-parasitoid networks). Then I compare changes in the network structure under a hypothetical nutrient enrichment scenario. In this chapter, I therefore address the following questions:

Q1: Do high levels of nutrients lead to less specialized insect networks? Because higher levels of nitrogen are expected to benefit generalist species (Pöyry et al. 2017), I predict that less specialized networks will be related to higher levels of nutrients. Q2: Do high levels of nutrients lead to more robust insect networks? Given that more interconnected networks are more stable to loss of species (Dunne et al. 2002). I predict that increase in generalism in the networks will increase the robustness of the network. Q3: Does the extinction of plant species under a hypothetical nutrient enrichment scenario, maintain the tendency of diminishing network specialization and increasing of network robustness? The vulnerability of the plant community to nutrient enrichment depends on species-specific differences in the optimum soil fertility and abundance of the plant species. Therefore some species may benefit while others are likely to be reduced in abundance (Stevens et al. 2016). Because rare species that tend to host more specialised insect are the most sensitive to nutrient enrichment (Suding et al. 2005, Vázquez et al. 2007, Fort et al. 2016), I expect an increase in generality and robustness of the network due to increase in connectance in my future scenario.

METHODS

Field sites

The study was undertaken in six areas in the city of Bristol in the south west of England. Four calcareous grasslands (The Downs, Crabtree, Ashton Court and Brandon Hill); and two acid grasslands (Troopers Hill, Oldbury Court Estate; Fig.2.1); both type of habitats have been reported

to be affected by nitrogen deposition (Maskell et al. 2010). Sites were selected to represent different urban grasslands around the city of Bristol where each field site was an independent replicate but also representative of the different soil fertility where species rich grasslands grow in the city. The management of all sites includes being mown in mid-July, although the hay is not removed from all sites.

Within each field site, two plots were chosen in areas with contrasting plant species richness (high vs low), this difference was assumed to be due to differences in soil fertility (see results) given the reported low plant species richness with nutrient enrichment (Peñuelas et al. 2013). The area of the plots varies from 450 m^2 to 600m^2, but any given pair of plots at a site were of equal size. In each plot, I established four permanent transects which ranged from 20-40m long, each running the whole length of the plot minus one meter at each edge (images of the layout plots within each field site are included in figures S. 2.1 a-f in the supplementary information)

Soil fertility

In each plot within each field site, I took 5 sub-samples from the top layer of soil for each plot within each field sites. Samples were collected using cores 5 cm diameter and 15 cm deep, following the protocol of the Countryside Survey (Emmett et al. 2008). Sub-samples were mixed, air dried and sieved using a 2mm sieve. The mixed air-dried samples were then sent to the Soil Analytical Chemistry laboratory at the Centre for Ecology and Hydrology where the following soil parameters were calculated: NH_4 and NO_3 (total organic nitrogen) total nitrogen (organic and inorganic nitrogen) organic carbon, soil moisture, phosphorous, pH and C:N (a summary of the methods used by the laboratory, is included in the Soil analysis section in the Supplementary Information).

Plant cover survey

In each of the four transects, five quadrats of 1m^2 were established every three to seven meters across the plot depending the length of the plot. All plants in each quadrant were identified to species (nomenclature for plants followed Stace 2010). The cover of each plant species was put into one of four categories as follows, using the methods of Gibson et al. 2007: Category 1 plants are rare, only present once to a few times in the whole quadrat, category 2 are present in high enough numbers to be seen easily (occupied < 10% of the quadrat area), category 3 could be seen throughout the whole quadrat (< 50% of the area) and category 4 are the most abundant (> 50% of the area). The system of Gibson et al. 2007 has shown to offer enough information of plant community when the aim is to test effects on the insect community (i.e. Macfayden et al. 2009)

or their ecological networks (Montoya et al. 2015). The simplicity of the systems of Gibson et al. 2007 by classifying plants only into four categories makes it easier and quicker to use in field than other classification systems with six or more categories (i.e. the method of Braun-Blanquet) that although offer a better detail, they also require more time in the survey which is a disadvantage when human resources are limited.

Quantifying ecological interactions

Every 14 days from May to July, I carried out a survey for herbivores in each plot, collecting externally feeding Lepidoptera and Coleoptera larvae, along with leaf miners in three of the five quadrants of $1m^2$; these being randomly chosen out of the five quadrats on each transect. Regardless, of the differences in the length of the transects or the plots, the total area sampled was the same for all 12 plots ($15m^2$). Insects were taken to the lab for rearing and kept in containers at room temperature, to rear them through to adulthood or to collect their parasitoids.

Flower visitors-plant interactions were recorded using timed surveys. These surveys consisted of walking for 20 minutes around the plot catching all flower visitors which touched the floral reproductive parts. Visitors were collected for identification and the plant they were visiting recorded.

Species identification:

Plants were identified in field using identification guides while flower visitor and herbivores were sent to the National Museum Cardiff and identified using traditional taxonomic methods (see acknowledgements). Given that parasitoids are challenging to identify using traditional taxonomy (Hrček and Godfray 2013), this group was sent to the Canadian Centre for DNA Barcoding where their DNA was extracted using automated glass fiber protocols (Ivanova et al. 2006) and a segment of the cytochrome *c* oxidase subunit I (COI) gene was amplified an sequenced using standard protocols (Smith et al. 2008a) to generate a "DNA barcode" (Hebert et al. 2003). Once the results of the DNA sequencing was finished by the laboratory, I performed the following bioinformatics analysis: I built a taxon identification tree using the neighbour joining protocol in the Barcode of Life Data Systems (BOLD ; Ratnasingham & Hebert 2007). BOLD assigns all sequences of sufficient length to barcode index numbers (BINs; Ratnasingham and Heber 2013) based on their similarity to specimens with already registered in the BOLD database. I examined sequences from within BINs and compared them to the reference collection.

I accepted taxonomic identifications suggested by BOLD when there was a match of 99-100% between our specimens and the identified record in BOLD with no contradictory information or

other similar sequences. When the match was between 97-98%, I recognized it as belonging to the same genus using the same criteria and for any lower match, I only accepted the family classification. Assigned names were then reviewed by an expert in parasitoid taxonomy (see acknowledgements) to confirm placements. Due to large number of samples and existing cost constraints, 278 out of 313 specimens were sent for analysis, these include all the rare specimens and 50% of the most abundant and common specimens reared from the most common host species.

Q1: Do high levels of nutrients lead to less specialized insect networks?

I built quantitative interaction matrices for each type of interaction (plant-flower visitor, plant-herbivore and herbivore-parasitoid network), using the frequency of interaction of flower visitors, herbivores and parasitoids. For each network, I calculated the following three topological network metrics which indirectly (connectance, interaction evenness) or directly (network specialization) measure the degree of specialization of the networks:

1. *Connectance (C_q):* number of links recorded in relation to the total number of possible links. The quantitative version of this metric is calculated by dividing the linkage density (mean number of interactions per species) by the total number of species in the network (Bersier et al. 2002). This metric is particularly useful when evaluating network structure as it is not affected by sampling effort (Tylianakis et al. 2010) and it gives some insight into the stability of the network;, as higher levels of connectance means higher redundancy and so likely higher stability (Dunne et al. 2002). In the context of this study, higher connectance could indicate more links realized per species, suggesting a higher number of generalist species. Formally the quantitative version of connectance it is calculated as followed:

$$C_q = \frac{LD_2}{S}$$

Where LD_q is the quantitative linkage density and S is the number of species in the network. Information on the calculation of LD_q is provided in the supplementary material.

2. *Interaction evenness (I.E.):* based on the Shannon index H (log2), this metric measures the equity of the links between the species; this metric can change even if species richness is unaffected (Bersier et al. 2002, Tylianakis et al. 2007). Its values range from 0-1 where 1 indicates maximum interaction evenness. This metric is calculated with the following formulae:

$$I.E. = \frac{\sum p_i \ log_2(p_i)}{log_2 N}$$

Where p_i is the proportion of the total number of species-host interactions (N) represented by interaction i (Tylianakis et al. 2007).

3. *Network specialization (H'_2):* is based on the Shannon index H but using natural log. It measures the deviation of observed interactions from the expected interactions under a maximum and a minimum specialization scenarios assuming that species interact with their partners in proportion to their observed frequency totals (Blüthgen et al. 2006). This a normalized metric unaffected by web size. It ranges between 0-1 where 1 indicates extreme specialization and 0 extreme generalization (Blüthgen et al. 2006). Mathematically is represented as follows:

$$H_2' = \frac{H_{2max} - H_2}{H_{2max} - H_{2min}}$$

Where H_2 is the observed network specialization and H_{2max} and H_{2min} are the maximum and minimum specialization, respectively, expected for the observed network. Calculous of H_2, H_2 max and H_2 min are included in the supplementary material.

Statistical analysis

I analysed the relationships between soil variables and each of the web metrics using a step-wise multiple regression in the package *MASS* (Venables and Ripley 2002). Model simplification is done by comparing values of Akaike Information Criterion (AIC), adding or removing variables to the model until the AIC did not decrease further (Venables and Ripley 2002, Cuthill 2014).

In the model, I considered both additive effect of the soil parameters and interactive effects between variables. Once the best model was selected, the significance of the explanatory power of each parameter on the metrics was tested by using a traditional F-test. In the particular case of connectance, which is highly influenced by species richness (Dunne et al. 2002), I included species richness as an explanatory factor in the model. All multiple regressions and F-test analyses were done using mixed effect linear models where place was considered as a random effect and soil fertility parameters (i.e. %nitrogen, %phosphorous, %carbon) as the fixed effects.

Presence of outliers was tested and until one outlier was removed models with and without outliers were tested and in all cases the model without the outlier was accepted. I consider this practice adequate since some particularities of some plots might not reflect the reality at a larger scale. For example, in some field sites insect resources particularly floral

resources were aggregated in < 1% of the plot clearly not representing the whole area or in other cases the network specialization was high, regardless the fact that herbivore species recorded in that network are generalists according to literature, mainly because of the low plant diversity. Normality of the linear models was tested using Shapiro's test of the residuals and homogeneity of variance was done visually. All measures of species richness were natural log transformed.

Q2: Do high levels of nutrients lead to more robust insect networks?

To calculate the specialization of the network while considering the ecological differences of each insect group, I calculated the web metrics separately for the three types of network (plant-herbivore, herbivore-parasitoid and plant-flower visitor). However, for the calculation of robustness given that in nature, species are interconnected forming a network of networks (Pocock et al. 2012) and given that the effects of nutrient enrichment on herbivores, parasitoids and pollinatros are plant mediated (Burkle and Irwin 2009, de Sassi et al. 2012a), I combined the three networks in a single network in which species from the second and third trophic levels are directly linked to the first trophic level (i.e. parasitoids are connected to the host plants of their prey). This way I created a "two" levels network where all species of the system are included in the network; this approach allows me to obtain a single and more representative measure of robustness (Carvalheiro et al. 2008).

By definition, robustness is a measure of the sensitivity of the network to collapse when extinction events occur. I calculated robustness by counting the number of species that remain in the network after a species is removed; this process is repeated until all species in the network are extinct (e.g. Memmott et al. 2004) . So, it assumes that a species only becomes extinct when all its resource species are removed from the network. Mathematically, this index is the integral of the function $y= 1-x^a$ that describes the curve of extinction where *x* is the amount of species than remain after species *a* was removed (Kaiser-Bunbury et al. 2010). A limitation of this method is the fact that it does not account for the "rewiring" of the interactions or the capacity of the species to interact with new species once all the interacting species are gone. Incorporating a model that includes rewiring and plant susceptibility to nutrient addition however, requires a more complex mathematical model like a Bayesian network model for which unfortunately there was not time neither human resources (see discussion chapter). However, since the aim of the chapter is to test a tendency to generalism due to high soil fertility being its effects on robustness a secondary question. This method although conservative is still a good approach as an exploratory analysis.

The removal of species is usually done randomly, by the abundance (the most abundant species first), by degree (the most or the least connected species first) or by species traits such as

body mass (Dormann et al. 2009). In the context of nutrient enrichment, it has been demonstrated that this causes non-random extinctions and for this reason, I used the Ellenberg indexes for nitrogen and pH (known as R) as well as plant abundance as criteria of order removal. The Ellenberg system is a well-known integer-based index, that indicate the optimum and tolerance of plant species to different environment factors such as temperature, light, soil pH (Hill et al. 1999). Ellenberg indexes have been widely used to detect and attribute the effects of elevated macro-nutrient supply on plant communities (e.g. Stevens et al. 2016; Hülber et al. 2017). Rarity is a common trait of species vulnerable to nitrogen enrichment (Suding et al. 2005). Also the structure of ecological networks is more vulnerable to a loss of rare species due to anthropogenic disturbance (Aizen et al. 2012). So far, only one other study has considered Ellenberg indexes as a criteria of species extinction in network analysis, thus Bähner et al. 2017 used moisture and temperature Ellenberg indexes to simulate extinction of trees in herbivore networks under a scenario of climate change.

Similarly, to Bähner et al. 2017, and by using the Ellenberg approach along with the data from each of the 12 plots, I simulated plant extinctions based on their fertility optimum (N Ellenberg index) and rarity, writing the code myself for this process. Species with the lowest tolerance to nitrogen where removed first but when two species had the same nitrogen optimum values, the species with the lowest abundance was removed first. This order of extinctions represents the realistic scenario where less nitrogen tolerant species and rare species go extinct first when levels of nitrogen increase in the system (Suding et al. 2005). Nitrogen optimum was estimated using the nitrogen Ellenberg index for British plants (Hill et al. 1999); rarity was calculated by using the percentage cover of each species from my plant surveys.

Statistical Analysis

To test whether soil fertility could explain variation in robustness. I carried out a stepwise multiple regression as explained in the previous question. Besides the soil fertility variables, I added the number of species in the network as an explanatory factor, this being done because robustness is influenced by the number of species in the network (Blüthgen 2010).

Q3: Do extinctions of plant species under a hypothetical nutrient enrichment scenario maintain the tendency of diminishing network specialization and increasing network robustness?

To test if expected changes in plant communities due to nitrogen enrichment lead to more generalist and more robust networks, I set a conservative hypothetical extinction scenario under

nutrient enrichment based on the predictions of changes in plant abundances under nitrogen deposition for the UK vegetation by Stevens et al. 2016. These authors made the predictions by measuring the responses of plants species to nitrogen enrichment such as their Ellenberg N index under different rates of nitrogen deposition in the UK. The authors found that under a low nitrogen deposition scenario some species would increase in abundance, others would become extinct and the majority would become less abundant. Although, this scenario is based on predictions for nitrogen deposition. I rather set my scenario as a "simulation of the cascading effects of the plant species responses to nutrient enrichment" and not specifically to nitrogen enrichment. This follows because I did not have site-specific histories of nitrogen deposition rate. I therefore set the responses of the plants using Ellenberg indexes of nitrogen and pH which represent the plant species optimum to soil fertility rather than directly to observed nitrogen levels at each site (Schaffers and Sýkora 2009).

To establish the inter-specific responses, I classified the plant species found in the plots into four different groups based on their average response to fertility and soil pH across the UK as represented by their Ellenberg N and R indices respectively (Hill et al. 1999). The Ellenberg index has been used before to predict changes in vegetation in response to macro-nutrient availability (see Stevens et al. 2010; Stevens et al. 2016). I also considered rarity since it is a consistent trait that predicts the probability of extinction under nutrient enrichment (Suding et al. 2005). The specific steps used to place each species into one of the four group of responses are explained in the selection criteria of classification of plant species in the supplementary material. The groups are as follows:

1) EXTINCT SPECIES: Species that will go extinct given their low abundance and preference for habitats with low soil fertility and high pH (i.e. *Cardamine pratensis* and *Plantago lanceolata*)

2) FUNCTIONALLY EXTINCT SPECIES: Rare species that due to their sensitivity to nutrient and acidification their populations will decrease to levels that make encounters with flower visitors and herbivores unlikely. Consequently, they will lose their functionality as resource species for other trophic levels (i.e. *Lotus corniculatus* and *Primula veris*).

3) TOLERANT SPECIES: Species that can maintain their abundance at levels able to maintain insect populations with change in functionality; these species are at their optimum at medium levels of fertility and acidification (i.e. *Centaura nigra* and *Trifolium prantese*).

4) NITROPHILOUS SPECIES: Species that are expected to increase their abundance under nitrogen enrichment (Stevens et al. 2016); these species grow well at high levels of nutrient and on acid soils (i.e. *Ranunculus repens* and *Holcus lanatus*).

THE RESPONSES OF OTHER TROPHIC LEVELS TO SIMULATED NUTRIENT ENRICHMENT

Primary consumers

Effects on the second trophic levels were based on the frequency of interactions. Species of herbivore and flower visitors with only one observation with extinct and functionally extinct plant species are likely to become extinct themselves and so were removed from the network. This accounts for the fact that in ecological networks rare interactions are more sensitive to extinction by anthropogenic disturbances (Aizen et al. 2012). In contrast, species that feed on functionally extinct plant species with more than one observation, were assumed to reduce their number of interactions due to a reduction in abundance of their host plant.

To simulate responses to diminishing host abundance, I conservatively deleted one observation from the "original" network. Under extinction events consumers are expected to either choose new hosts (rewiring) or redistribute their feeding interactions by selecting another resource from its original resources (Gilljam et al. 2015). Since rewiring is hard to predict due to the inter-specific changes in attractiveness resulting from nutrient enrichment (see Throop & Lerdau 2004; Burkle & Irwin 2010; de Sassi *et al.* 2012b; Hoover et al. 2012), I used a redistribution of interactions approach by adding the lost interactions of consumer species to the most visited species from the group of nitrophilous plants. In this scenario, species feeding on extinct species or a species feeding on functionally extinct plant and with less than two observations were removed from the network while lost interactions where added to nitrophilous species if such interaction existed in the original network (Fig. 2.2).

Secondary consumers

The responses of the secondary consumers (the parasitoids) were based on the changes in the herbivore community. Species that were only observed in herbivores that went extinct were predicted to go extinct too. Since each interaction in a network represents an individual, the removal of an interaction represents a decline in the abundance of prey for parasitoid, so a decline in parasitoids abundance is therefore expected. The interaction loss between herbivores and tolerant plant species and herbivore were also removed in the herbivore-parasitoid network. Finally, as done with the herbivore community, the links between parasitoids and herbivore that became extinct were added to a pre-existing interaction between the preferred herbivore when feeding on a nitrophilous species.

Network Analysis

To compare changes in the network specialization and robustness. I calculated connectance, interaction evenness, network specialization and robustness using the same methods as used in question one. Thus, I calculated robustness using the Ellenberg removal approach, however I also compared the robustness under a random extinction scenario for the two networks. This was done as a null model approach to test the robustness of the network under factors other than nutrient enrichment, for which the prediction of a future trend is hard to define (Fukami et al. 2001).

Statistical Analysis

I compared differences in network structure between the 12 observed networks (the field data) and their matched simulated networks using a paired t test. Based on my earlier predictions I tested for an increase (one tail test) in connectance, interaction evenness, network specialization and robustness in the predicted networks. Finally, I tested for a decline in the number of species of plants, herbivores, flower visitors and parasitoids for the original and simulated networks also a paired t test.

RESULTS

A total of 50 species of plants and 205 species of insects were collected from the 12 plots: the insects consisted of 95 species of flower visitor (n = 575), 59 species of herbivores (n=892) and 51 species of parasitoid (n= 312); see appendix 1 for the species list Table 1-4. The use of plant species richness as a proxy of soil fertility when choosing the plots proved to be a good approach as I observed differences in soil fertility within and between field sites (Supplementary information, Table S. 2.1). Network representations from places with high and low levels of nutrients are shown in the figure 2.3.

Q1: Do high levels of nutrients lead to less specialized insect networks?

Connectance: In the flower visitor's networks, there was a negative relationship between species richness and organic carbon, this being in contrast with positive relationships between connectance and total nitrogen, organic nitrogen, C: N and soil moisture (Table 2.1). However, in herbivore networks an increase in connectance was explained by interactive levels of phosphorous and organic carbon (-CO * -P) and a decrease in species richness (Table 2.1). Finally, in parasitoid networks organic nitrogen was negatively related to connectance when considering

the effects of organic carbon, species richness and pH (out of these, only species richness and pH have a significant explanatory power; Table 2. 1).

Interaction evenness: In flower visitor networks phosphorous significantly explained a decrease in interaction evenness when controlling for a non-significant positive effect of total nitrogen. In herbivore networks a higher interaction evenness was marginally associated only with organic carbon (Table 2.1) while in parasitoid networks total nitrogen, phosphorous C: N and soil moisture were significantly related to lower values of interaction evenness when considering soil organic carbon (Table 2.1).

Network specialization: In the flower visitor networks a decrease in network specialization was associated with higher levels of soil moisture when accounting for the effect of phosphorous (Table 2.1). In herbivore networks, phosphorous marginally explained a decrease in H'_2 when considering the effects of carbon and the interactive effects between phosphorous and carbon (Table 2.1) while in parasitoid networks less H'_2 was explained by total Nitrogen levels when considering the effect of pH (Table 2.1).

Q2: Do high levels of nutrients lead to more robust insect networks?

Higher levels of robustness were associated with higher levels of organic nitrogen and soil moisture (0.03±0.004, $F_{1,3}$=5.18, p=<0.0001; 0.047±0.041, $F_{1,3}$=6.49, p=0.0001, respectively, Table 2.2.).

Q3: Do extinctions of plant species under a hypothetical nutrient enrichment scenario maintain the tendency of diminishing network specialization and increasing network robustness?

In all three interaction networks the number of consumer species was significantly lower in the network subject to simulated eutrophication (Fig. 2.4 and fig. 2.5). In plant-flower visitor networks, the simulated networks have a significantly higher connectance and network specialization with no change in interaction evenness (Fig. 2.6 & Table 2.3). In plant-herbivore networks, connectance significantly increased in the predicted networks while network specialization and interaction evenness did not significantly change (Table 2.3). In herbivore-parasitoid networks there was no significant difference between the original and the simulated network for any of the network specialization metrics (Table 2.3).

When species were removed, ordered by each species' sensitivity to nutrient enrichment and rarity, there was no significant difference between the original and the simulated network

(t=-0.34, d.f.=11, p=0.74). However, when the removal was done randomly, the robustness of the simulated networks was significantly lower (t=2.38, d.f.=11, p=0.01; Fig. 2.7).

DISCUSSION

My field data showed that the various soil fertility parameters affect the structure of the interaction networks. In general, and as predicted, I observed a tendency for loss of specialization of the networks and an increase in robustness with high soil fertility and low pH, this being due to the decrease in plant species richness. The main nutrients affecting network structure vary with the type of ecological network. Thus, in plant flower visitor network, phosphorous and soil moisture are most important, in plant-herbivore networks it is carbon that is important and in herbivore-parasitoid networks, nitrogen. The simulation of future eutrophication impacts showed that these changes are expected to continue into the future. In this section, I first consider the limitations of my study, then put the results into a more general context.

Limitations

There are two main limitations to this work. First, the sample size is relatively low as I compared 12 grasslands, four of which were acid grasslands, and so the statistical power is low. That said, some of the effects of soil fertility were detectable even a small sample size, although it is likely that subtle effects will have been missed. While small scale, the study is novel in that it asks about the effect of fertility on three types of ecological network simultaneously. The second limitation is that the predictive models need refinement. I estimated a loss of one individual insect feeding on low enrichment tolerant species when I simulated the response of consumers to a decline in abundance of one of their host plants. However, at a population level, the loss is likely to be higher but also hard to predict although desirable for a more realistic approach (Gilljam et al. 2015). Moreover, I did not consider that the level of nitrogen deposition and nitrogen emissions have decreased in the UK since Stevens *et al.* 2006 modelled the responses of the British habitats. However, I did include the effects of acidification which is a major driver of changes in vegetation (Stevens et al. 2010b).

Q1: Do high levels of nutrients lead to less specialized insect networks?

In natural conditions the interaction of nutrients is complex, and it is obvious that looking at one nutrient in isolation cannot explain the observed patterns. Soil properties, including the ratios of nutrients, influence the efficiency of nutrient uptake by plant species, and trigger competitive

interactions that change the plant community composition (Harpole et al. 2016). I found that although several nutrients are needed to explain the decrease in the specialization of the networks, the main nutrients that affect network structure vary with the type of ecological network.

In plant-flower visitor network, phosphorous and soil moisture are the main parameters, in plant-herbivore networks it is carbon that is important and in herbivore-parasitoid networks, nitrogen. Although, these results need to be interpreted with caution due to low statistical power and the biogeochemical correlations between macro-nutrients and soil conditions, they are supported by published work. For example, Harpole et al. (2016) showed that nutrients such as phosphorous triggered different competitive mechanisms and that these led to a loss of plant species, along with an increase in dominance of some species. The negative impacts of phosphorus on plant species, have been pointed out several times before and some authors have even considered it as a more important driver of species loss in semi-natural grasslands than nitrogen (Ceulemans et al. 2011, Ceulemans et al. 2013).

Natural variation in pH and soil depth probably led to small-scale variation in soil nutrients mainly phosphorous. In the case of plant-herbivore networks, it has been observed that under nutrient enrichment some plant species tend to have softer leaf tissues as C:N ratio decreases resulting in greater palatability (Throop and Lerdau 2004, Kurze et al. 2017). Thus the positive correlation between lower soil C:N and increased above-ground biomass of plants with lower C:N is consistent with an indirect effect on network structure resulting from spatial variation in soil fertility. That said, I did not observe a relationship between network structure and either soil nitrogen or soil C:N ratio but instead organic carbon was important in the plant-herbivore networks. Since leaf traits were not measured in this study I cannot rule out the possibility that inter and intra-specific changes in leaf traits might correlate with differences in network structure and that soil measurements were simply an insensitive correlate of these above-ground relationships. An interesting result was the interactive effect between carbon and phosphorous which led to more interconnected herbivory networks. While independently both elements decrease connectance, when they act together, they increase connectance. This shows the interactive effects of nutrients along with the real challenges of working in this area, especially in the field.

In the herbivore-parasitoid network, the observed influence of nitrogen on the generalization of the network could be due to the increase in the abundance of herbivores. Parasitoids respond to abundance of herbivores (Stiling and Moon 2005, de Sassi and Tylianakis

2012) so this could attract more parasitoids which are functionally a generalist group. Moreover, under nitrogen enrichment some plants switch from physical defences to chemical defences and some of these are volatiles which attract parasitoids (Throop and Lerdau 2004). This plant facilitation for herbivores location could potentially increase the number of herbivore species attacked and so increase the connectance of the network.

Q2: Do high levels of nutrients lead to more robust insect networks?

My results showed that the plots with higher levels of soil moisture and total organic nitrogen have a higher robustness. Soil moisture is important for the oxidation-reduction reaction in soil that allows the assimilation of nutrients by plants (i.e. Bohrerova et al. 2004 and Dwire et al. 2006) . With different nutrients explaining the network structure of the different biotic interactions, it makes sense that soil moisture was correlated to higher network robustness since different nutrients promoted generalism in the network. The fact that total organic nitrogen has also an explanatory power might be due to the approach I used to remove species which is based on N Ellenberg. However, the N Ellenberg index is not exclusively related to nitrogen, but with fertility in general (Schaffers and Sýkora 2009). In this sense, although my results show that organic nitrogen can explain the increase in robustness, I will take a conservative interpretation and attribute it to a general increase in fertility with no reference to one specific nutrient.

Q3: Do extinctions of plant species under a hypothetical nutrient enrichment scenario maintain the tendency of diminishing network specialization and increasing network robustness?

As reported from studies on changes in the distribution of Lepidoptera in relation to nitrogen deposition (Pöyry et al. 2017), I found a tendency to increased generalism in the networks as fertility increased. My simulations led to more interconnected plant-flower visitor and plant-herbivore networks, but with lower robustness to random extinction events. The insect species which will be the most sensitive to eutrophication are specialist or monophagous insect species feeding on non-nitrophilous plant species (Pöyry et al. 2017) from low fertility sites (Öckinger et al. 2006). Losing these species from the network will (as observed here) increase the connectance of the network. It could be argued that this increase is an artefact because rare species establish fewer interactions within the networks (Vázquez et al. 2007, Fort et al. 2016) and so removing them increases the connectance by default. However, the simulations were based on estimates of the soil fertility and pH optimum for each species and in this sense the results are realistic responses of species to nutrient enrichment.

Although, insect species richness decreased in all networks, the robustness of the simulated networks under the removal approach of nitrogen and rarity was no different than the robustness of the original networks. This could be due to the removal of the most sensitive species in the low nutrient enrichment scenario, species which tend to be more specialized and so their removal in the original networks when calculating robustness had relatively little effect. In contrast though, the random removal did lead to a significant decrease in robustness. It is well known that species richness confers higher robustness to random extinctions (Memmott et al. 2004, Kaiser-Bunbury et al. 2010) and in this sense the decrease in robustness of the less species rich simulated networks is not surprising. Nevertheless, different outcomes in the two approaches shows that the simulated networks were not less robust to species loss due to nitrogen enrichment than the original networks (when species are removed in order of their fertility optimum). However, simulated networks are less robust than the original networks to random loss species. This result shows that in a future scenario of nutrient enrichment networks would loss the buffering effect of species richness to other types of disturbance diminishes.

CONCLUSIONS

One of the main outcomes of this study is that it is important to consider soil nutrients collectively rather than in isolation. Thus, nutrients can interact and so have different effects in different contexts and they also affect different components of the community in different ways as seen here. The simulation of nitrogen enrichment, while simplistic, illustrates clearly that impacts on the plant community will cascade upward through the community and affect other groups indirectly, for example flower visitor and parasitoids. Moreover, the simulation is not a computational simulation where networks were created by mathematical parameters, but a simulation based on real data and therefore providing more realistic predictions. Studies on changes of vegetation and Lepidoptera due to nitrogen deposition have been undertaken in several European countries (i.e. Öckinger *et al.* 2006 Maskell *et al.* 2010; Stevens *et al.* 2010a). Wallis, DeVries & van Swaay 2017 even proposed the creation of indices of nitrogen optimum for Lepidoptera species. However, we need to develop predictions that include more species groups and other environmental factors, and above all, we need predictions on the effects of these factors on the functionality of ecological systems. This would enable both a better management and a better conservation of our natural resources.

Table 2.1. *Results of the best model selection by the step wise regression model analysis for specialization of the network.*

NETWORK	METRIC	PARAMETER	ESTIMATE ± STD. ERROR	t VALUE	Pr (>|t|)	F VALUE	Pr (>F)
Flower visitors	*Connectance*	Organic carbon	-0.032±0.007	-4.48	0.01*	24.64	**0.008***
		Total nitrogen	0.306±0.075	4.1	<0.0147*	1.51	0.286
		Organic nitrogen	0.017±0.002	9.52	0.007*	9.79	**0.03***
		C: N	0.014±0.004	3.77	0.02*	25.9	**0.007**
		Soil moisture	0.017±0.002	8.72	0.001*	387.768	**<0.0001***
		Species richness	-0.008±0.0006	-12.56	<0.0002*	157.77	**<0.0002***
	Interaction evenness	Phosphorous	-0.003±0.001	-4.07	0.004*	15.89	**0.004***
		Total nitrogen	0.129±0.074	1.74	0.12	3.02	0.12
	Network specialization	Phosphorous	0.005 ± 0.002	2.63	0.03*	2.91	0.126
		Soil moisture	-0.032 ± 0.01	-2.97	0.017*	8.86	**0.017***
Herbivores	*Connectance*	Organic carbon	-0.02±0.01	-2.82	0.106	1407.99	**0.0007***
		Phosphorous	-0.02± 0.002	-9.95	0.01	183.17	**0.005***
		P: Organic C	0.004±0.002	15.14	0.004	46.227	**0.0043***
		Species richness	-0.004±0.001	-2.62	0.12	229.3	**0.021***
	Interaction evenness	Organic carbon	0.04±0.016	2.50	0.05*	6.28	**0.05***
	Network specialization	Phosphorus	-0.013±0.004	-2.89	0.06	0.179	**0.70**
		Organic nitrogen	-0.031±0.019	-1.65	0.19	1.98	0.25
		Organic carbon	0.001±0.00006	2.65	0.07	7.02	0.07
Parasitoids	*Connectance*	Organic nitrogen	-0.016±0.004	-3.71	0.02*	14.87	**0.02***
		Organic carbon	-0.01±0.006	1.58	0.188	1.21	0.33
		pH	0.023±0.011	2.08	0.105	6.31	0.065
		Species richness	-0.006±0.001	-6.86	0.002*	47.12	**0.002***
	Interaction evenness	Total nitrogen	-0.9±0.23	-3.86	0.06	292.54	**0.003***
		Phosphorous	-0.001±0.001	-3.89	0.06	24.49	**0.04***
		Organic carbon	0.01±0.02	5.77	**0.03***	6.39	0.12
		C:N	-0.05±0.01	-5.44	**0.03***	22.98	**0.04***
		Soil moisture	-0.007±0.002	-4.9	**0.04***	24.03	**0.04***
	Network specialization	Total nitrogen	-1.7±0.49	-3.44	0.02	22.14	**0.005***
		pH	0.10±0.07	1.51	0.19	2.29	0.19

Table 2.2 *Results of the best model selection by the step wise regression model analysis for the robustness of the insect communities in the plant-primary consumer-secondary consumer. Robustness* was calculated by measuring the remaining species after the removal of one plant species. Plant species order of removal was according with the nitrogen optimum and rarity.

PARAMETER	ESTIMATE ± STD. ERROR	t VALUE	Pr (>|t|)	F VALUE	Pr (>F)
Dry	0.0475±0.004	11.138	0.007*	6.499	**<0.0001***
Organic nitrogen	0.0316±0.004	7.503	0.049*	5.188	**<0.0001***

Table 2.3. *Differences between original network and the simulated network*. Mean difference was calculated by subtracting the value of the simulated network to the original network

Metric	Mean difference	t	d.f.	p
Plant-flower visitor Network				
Flower visitor species richness	0.4834	7.15	11	**<0.0001***
Connectance	-0.1129	-2.146	11	**0.027***
Interaction evenness	0.0233	1.49	11	0.164
Network specialization	-0.1789	-2.53	11	**0.013***
Plant-Herbivore Network				
Herbivore species richness	0.1636	3.16	11	**0.004***
Connectance	-0.0281	-3.07	11	**0.005***
Interaction evenness	0.0116	0.70	11	0.49
Network specialization	0.0002	0.02	11	0.51
Herbivore-Parasitoid Network				
Parasitoid species richness	0.3631	2.39	8	**0.021***
Connectance	-0.1266	-1.5	8	0.085
Interaction evenness	-0.0001	-0.01	8	0.494
Network specialization	-0.075	-1.11	8	0.139
Plant-Primary Consumer-Secondary Consumer Network				
Plant species richness	0.1377	6.32	11	**<0.0001***
Random robustness	0.1473	2.38	11	**0.018***
Nitrogen & rarity robustness	-0.0074	-0.34	11	0.368

Table 2.4. *Results of the best model selection by the step wise model analysis for the relationships between soil fertility and differences between original and simulated networks.* Only metrics that were significantly different between the two metrics were tested.

NETWORK	METRIC	PARAMETER	ESTIMATE ± STD. ERROR	t VALUE	Pr (>|t|)	F VALUE	Pr (>F)
Flower visitor	*Flower visitor richness*	Plant richness	0.5±0.26	1.93	0.08	3.74	0.08
	Connectance	Intercept	-0.11±0.05	-2.14	0.05*	NA	NA
	Network specialization	pH	0.11 ± 0.06	1.81	0.09	3.305	0.099
Herbivores	*Herbivore richness*	Plant richness	0.90±0.11	7.80	<0.0001*	60.55	**<0.0001***
		Soil moisture	-0.005± 0.004	-1.43	0.19	2.45	0.15
		Total nitrogen	0.1±0.07	1.36	0.21	1.86	0.21
	Connectance	Species richness	-0.008±0.001	-9.515	<0.0001*	90.52	**<0.0001***
		Organic nitrogen	0.003±001	1.54	0.16	13.91	**0.005**
		Soil moisture	-003±0.002	-1.53	0.16	0.77	0.403
Parasitoids	*Parasitoid richness*	Soil moisture	-0.05±0.015	-3.35	0.01*	12.84	**0.012**
		Total nitrogen	0.367±0.28	1.31	0.23	1.722	0.23
Plant-primary -secondary – consumer	*Plant richness*	Intercept	0.137±0.022	6.314	<0.0001*	NA	NA
	Robustness to random extinction	Organic carbon	0.006±0.001	4.103	0.002*	17.61	**0.002**
		Species richness	-0.08	0.021	-3.98	15.91	**0.003**

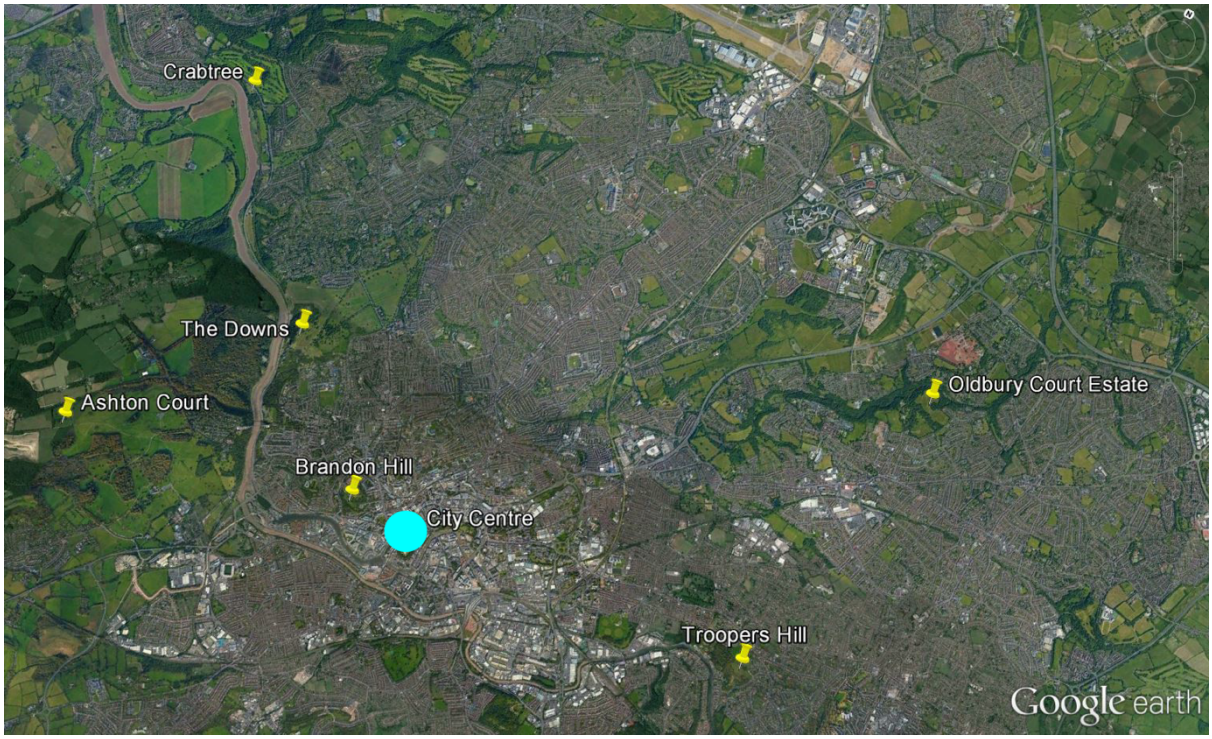


Figure 2.1. *Locations of the pairs field sites in Bristol*. field sites are marked by yellow pins. The blue circle indicates the city centre.

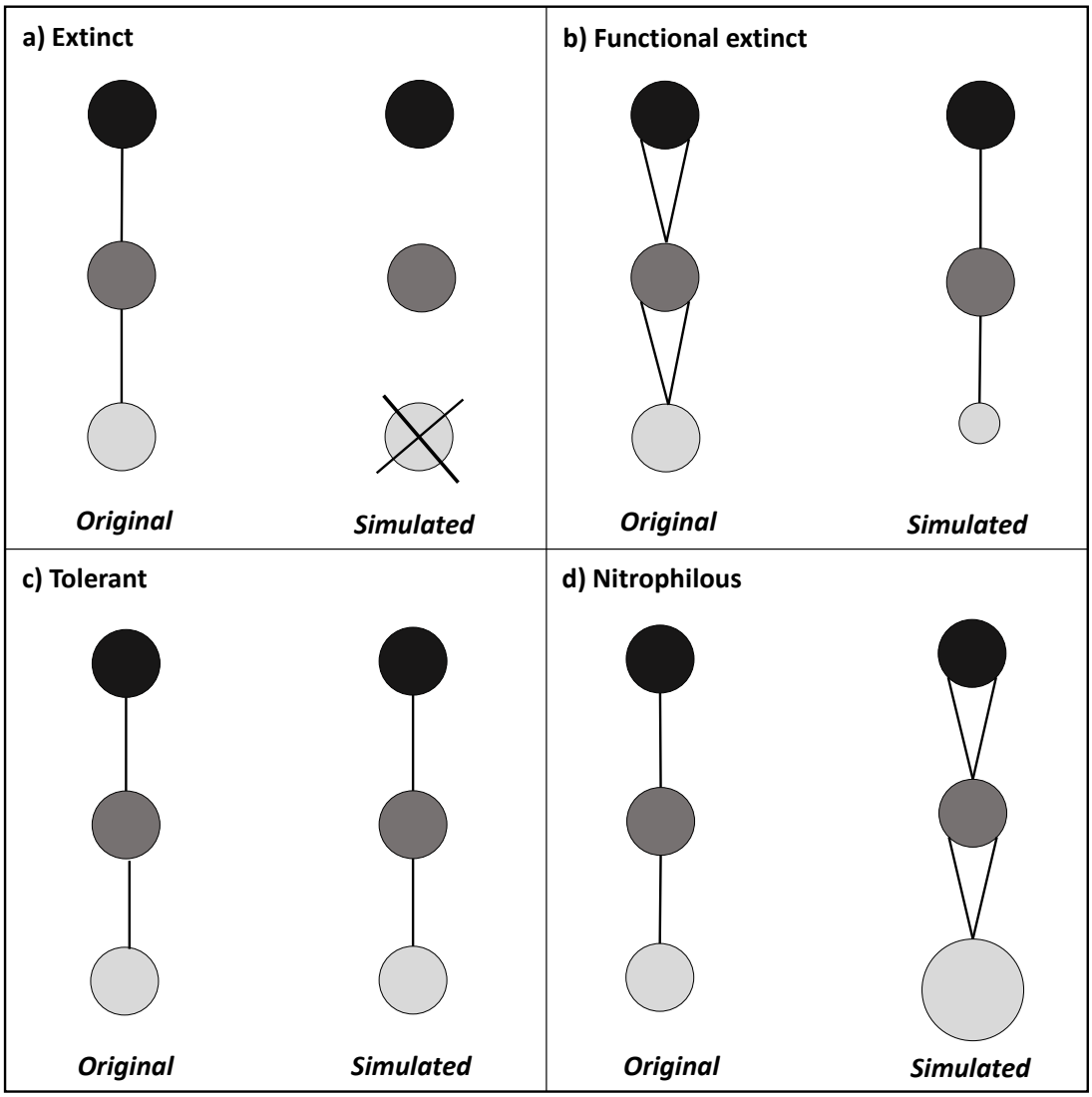


Figure 2.2 *Example of the cascading effects on individuals of consumer species of one plant species from each plant response group.* Circles represent species the position is according to the trophic level of the species (producers-primary consumers, secondary consumers. The x represent extinction. Interactions unique of an extinct species was removed. Functional extinct species are those expected to decrease their abundance, so the number of species and individual of species feeding on such species. Tolerant species are expected to remain the same while nitrophilous species will become more abundant attracting more consumers.

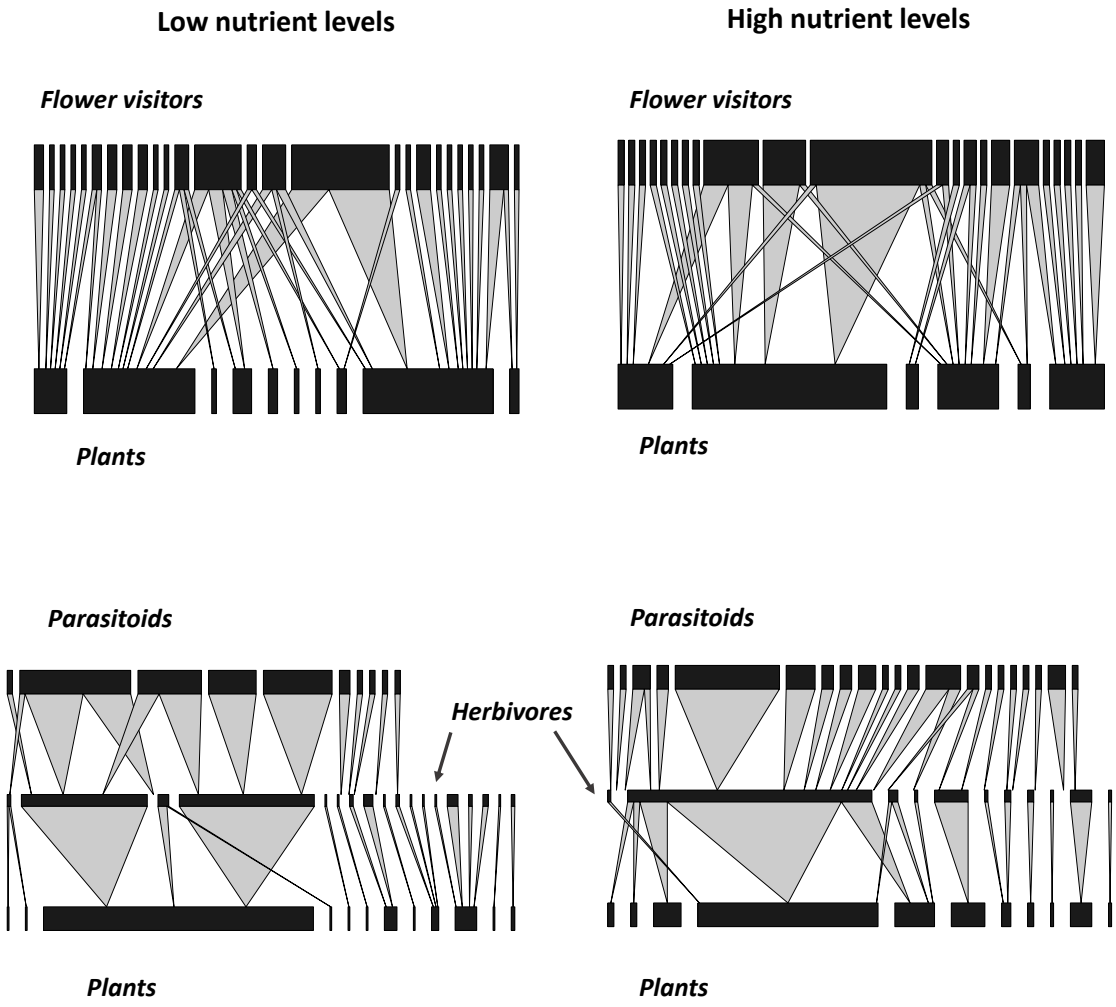


Figure 2.3. *Network from places with low nutrient levels versus high nutrient levels.* An example of the plant-flower visitor network and plant-herbivore-parasitoid from a plot with low nutrient levels and a plot with high levels of nutrient or soil fertility. From down to top plant species are represented in the first level of the networks, flower visitors and herbivores in the second level and parasitoids in the third level as indicated in the figure. The width of each rectangle corresponds to the number of links that each species establishes with species from the opposite trophic level.

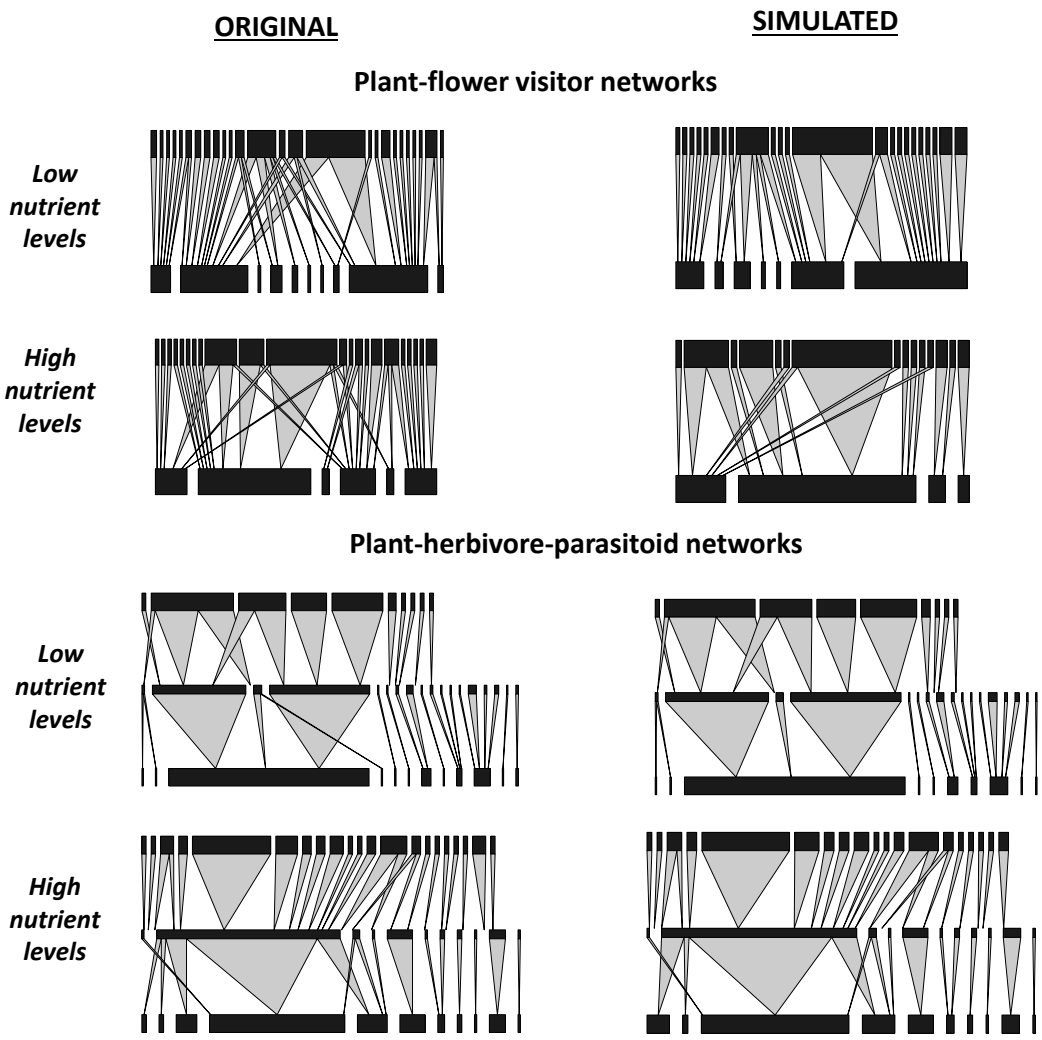


Figure 2.4 *Original vs simulated networks places with low nutrient levels and high nutrient levels.* An example of the changes of the plant-flower visitor network and plant-herbivore-parasitoid from a plot with low nutrient levels and a plot with high levels of nutrient level or soil fertility after the removal species under a nutrient enrichment scenario. Original networks are illustrated at the right and the simulated networks at the left. From down to top plant species are represented in the first level of the networks, flower visitors and herbivores in the second level and parasitoids in the third level. The width of each rectangle corresponds to number of links that each species establishes with species from the opposite trophic level.

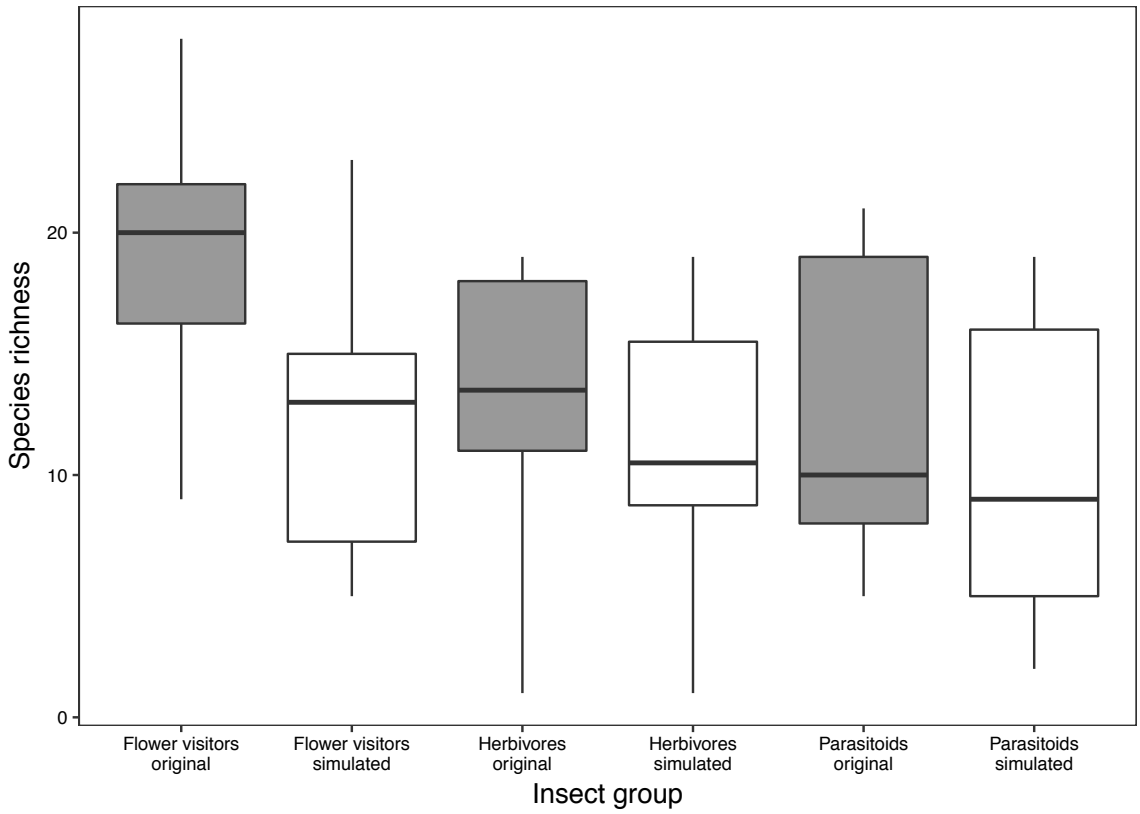


Figure 2.5. *Comparison of species richness of herbivores, parasitoids and flower visitors between the original and the simulated networks.* Original networks are shown in grey and white boxes represent the simulated networks.

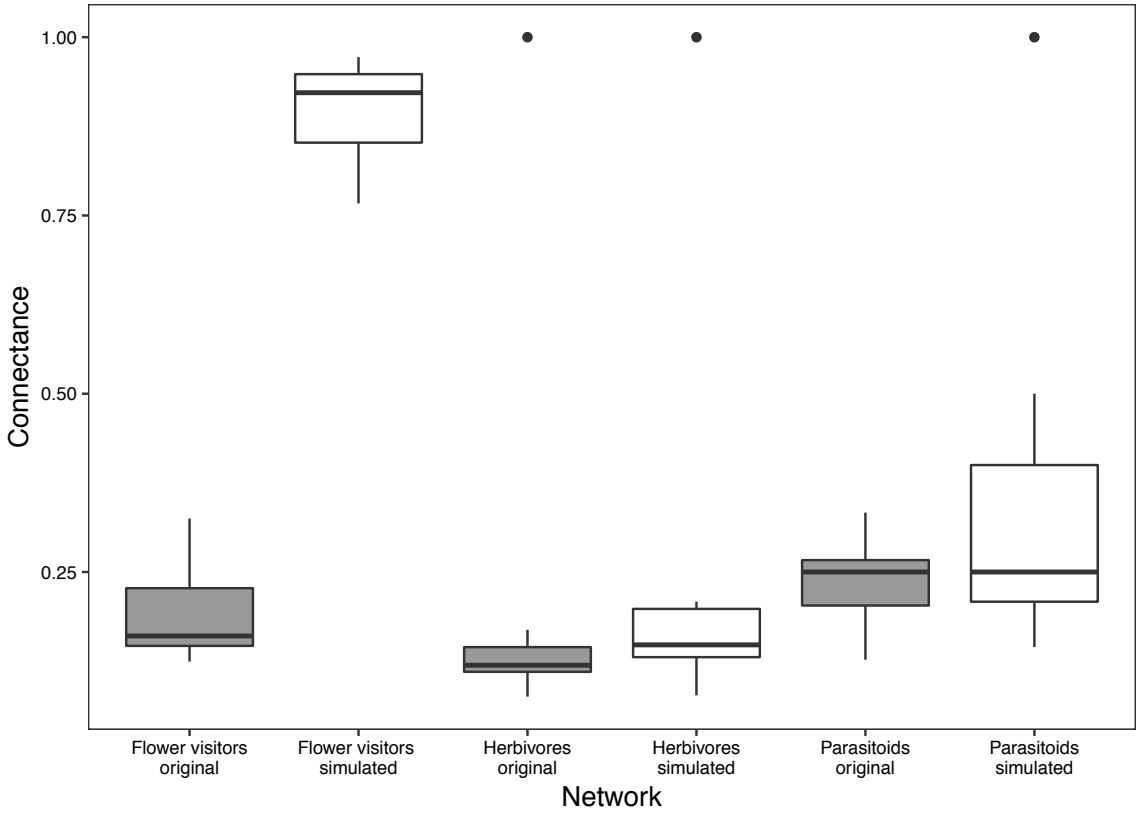


Figure 2.6. *Differences in connectance of the different groups between the original and the simulated networks.* Original networks are indicated in grey and white boxes represent the simulated networks.

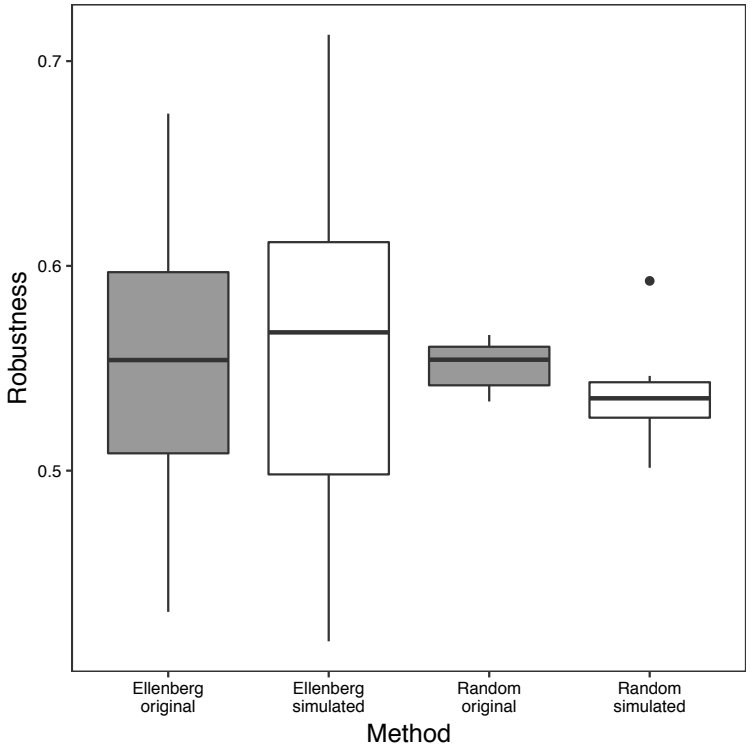


Figure 2.7. *Network robustness for the original and the simulated networks under two methods of species removal***.** Original networks are shown in grey and simulated networks are shown in white. The x-axis shows the method used for the removal of the plant species: *Ellenberg* refers a removal based on nutrient optimums and rarity; and *Random* to a random removal.

SUPPLEMENTARY INFORMATION

Soil analysis methods

Methods were provided by the Centre for Ecology & Hydrology, Lancaster Environment Centre under the code NEC 05787 T03 Methods. The soil samples were air dried so a dry weight at 105°C correction was applied to the results to account for moisture.

Soil moisture (%Dry): 1 g of airdried soil was weighted into dried, cooled & pre-weighted crucibles. Crucibles were dried at 105°C for 3 hrs, cooled and weighed again. Percentage of dryness was then determined through calculation of the loss on ignition (LOI) or change in mass of the sample after heating the sample.

Total organic nitrogen (TON)*:* It refers to the percentage of nitrate (NO_3) and nitrite (NO_2) in soil. These compounds were extracted by adding 100 ml of 6% potassium chloride (KCL) as reagent; and mixed using an end over end shaker for 30 min. The sample were filtered through a Whatman 44 filter paper to separate the soil from the filtrate. Finally, the percentage of TON was calculated by analysing the filtrate on a discrete analyser AQ2 by SEAL Analytical.

Total nitrogen**:** a ball milled soil air dried is oven dried at 105 °C (±5°C) for a minimum of 3 hours, cooled, and weighted on a 6 place micro-balance. Total nitrogen was calculated using an Elementar Vario instrument which is an instrument that works on the principle of oxidative combustion and thermal conductivity detection. In simple words, in the elementar vario the sample is burned at a temperature of 1800 and the gas produced is separated into different gas products of known chemical form. Then through different chemical reactions, the element of the interest (i.e. nitrogen or carbon) is separated from the gas products. Finally, the concentration of the element is calculated by measuring the electrical signal of the sample which is proportional to the concentration of the element in the sample. The calibration of the elementar Vario was checked by working standard (Acetanilide) with a concentration of 10.4% total N and the data corrected (factored) against this value.

Organic carbon**:** a ball milled soil previously air dried is repeatedly acidified with 1-2 ml of 3 M (HCL) and dried until the reaction with inorganic carbon ends. The excess of acid is evaporated off in a fume cupboard. Samples are oven dried at 105 °C (±5°C) for a minimum of 3 hours, cooled and 20 g of sample is weighted on a 6 place micro-balance. Carbon content as total nitrogen was calculated using an Elementar Vario instrument but with calibration checked using a working standard (Acetanilide) with a concentration of 71.1 % and total C and data corrected (factored) against this value.

Phosphorous: this was calculated following the method of Olsen Phosphorous in soil. 2 gr. In which phosphorous is extracted by adding to 2gr sample of air-dried soil, 40 ml Olsen´s reagent (0.5 M $NaHCO_3$ at pH 8.5). The sample is filter through a Whatman 44 filter and the filtrate is analysed on a Seal Analytical AA3 segmented flow analyser.

pH: 10 cm_3 of fresh soil is mixed with 10 ml of ultra-pure water in a 50ml beaker, mixed vigorously into a paste, topped up to the 40ml mark, stirred well and allowed to settle for 20 minutes. The sample is then tested with a pH electrode calibrated using three buffer solution at pH 4, pH7 and pH 10.

C:N ratio: organic carbon divided into total organic nitrogen.

Connectance-calculous of linkage density (LD_2)

The quantitative version of linkage density considers the diversity of biomass going from one species to another species from the other trophic level (Bersier et al. 2002). Diversity of the biomass from a species *i* first trophic level to species k from the second trophic level is represented as H_N while the diversity of biomass from a species *j* of the first trophic level to species k of a species from the second trophic level is represented as H_P. H_N and H_P are calculated as follows:

$$H_{N,k} = -\sum_{i=1}^{S} \frac{b_{ik}}{b_{\cdot k}} log_2 \frac{b_{ik}}{b_{\cdot k}}$$

$$H_{P,k} = -\sum_{j=1}^{S} \frac{b_{kj}}{b_{k\cdot}} log_2 \frac{b_{kj}}{b_{k\cdot}}$$

Where *k* represents the species, $b_{\cdot k}$ refers to the sum of the interactions of species from the second trophic level and $b_{k\cdot}$ is the sum of the interaction of species of the first trophic level. The metric assumes that biomass of all species is identical. This assumption is included in the calculous by calculating the reciprocals of H_N and H_P as follows:

$$n_{N\cdot k} = \begin{cases} 2^{H_{N,k}} \\ 0 \end{cases} if\ b_{\cdot k} = 0$$

$$n_{P\cdot k} = \begin{cases} 2^{H_{P,k}} \\ 0 \end{cases} if\ b_{k\cdot} = 0$$

Finally, the he quantitative version of linkage density represented as LD_2 is calculated as follows:

$$LD_2 = \frac{1}{2}\left(\sum_{k=1}^{s}\frac{b_{k\cdot}}{b\,\cdot\cdot}2^{H_{P,k}} + \sum_{k=1}^{s}\frac{b_{\cdot k}}{b\,\cdot\cdot}2^{H_{N,k}}\right)$$

Network specialization-calculation of the H2, H2 max and H2 min

As explained in the main text network specialization or H'_2 was created by Blüthgen *et al.* in 2006 and is based on the shannon index and mathematically is expresed as follows:

$$H_2' = \frac{H_{2max} - H_2}{H_{2max} - H_{2min}}$$

Where H_2 is the observed specialization network and H_{2max} and H_{2min} are the maximum and minimum specialization, respectively, expected for the observed network.

H_2 is calculated by adapting the Shannon entropy by summarizing the proportion of realized interaction of the first trophic level and the proportion of the realized interaction of the second trophic level. It is calculated as follows:

$$-\sum_{i=1}^{r}\sum_{j=1}^{c}(pij \cdot lnpij)$$

Where *i* represents the first trophic level and *j* the second trophic level and *pij* is the proportion of the frequency of interactions between specie *i* and species *j* in relation to the total number of interactions.

To be able to standardize H2 between 1 and 0 for extreme generalization versus extreme specialization, maximum H2 and a minimum H2 (H_{2min}) are calculated (H_{2max}).

H_{2max} is calculated with the formulae:

$$H_{2max=} -\sum_{i=1}^{r}\sum_{j=1}^{c}(qiqj \cdot lnqiqj)$$

Where *qi* is the maximum number of interaction of species of the first trophic level and *qj* the maximum number of interactions of species of the second trophic level.

The calculous of H_{2min} is less straight forward since although theoretically the value may be close to zero mathematically can differed since the value is calculated from integer values. For this the authors used a heuristic solution based on the method described by Patefield 1981.

In short, they adapted the formulae as follows:

$$H_{2min=} - sum(p(i,j) * \log p(i,j))$$

Where p means probability and *i* specie of the first trophic level and *j* species from the second trophic level.

Criteria of classification of plant species

Steps followed to classified plant species into the four categories of response to the simulation of nutrient enrichment.

If species has a value of:

a) N Ellenberg <4, R Ellenberg >6 and cover < = 1% ……. *extinct.*
b) N Ellenberg >1 but <4, R Ellenberg is >6 and cover >1% ……. *functionally extinct*.
c) N Ellenberg between 4-5, R Ellenberg is between 4-5 and cover <1% …… *functionally extinct.*
d) N Ellenberg between 4-5, R Ellenberg is between 4-5 and cover >1% ……. *tolerant*.
e) N Ellenberg >5, R Ellenberg is >5 cover >=1% …… *nitrophilous*.

Soil fertility.

Supplementary information table. S.2.1. *Results of the analysis of the soil fertility for each of the plot.* The letter corresponds to the name of the place and the number indicates one of the two plots within the same field site. A: Ashton, B: Brandon Hill, C: Crabtree, D: The Downs, O: Oldbury Court and T: Troopers Hill.

Factor	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
Soil moisture	96.6	91.9	95.2	95.6	95.2	89.8	94.4	93.9	96.4	97.1	94.8	96.8
Total organic nitrogen	3.9	6.9	4.2	6.5	2.5	8.7	2	2.6	3.1	4.3	5.7	2.2
Total nitrogen	0.36	0.58	0.55	0.43	0.42	0.48	0.62	0.74	0.49	0.63	0.39	0.6
Organic carbon	3.71	6.58	5.72	4.54	5.11	4.82	6.4	6.37	4.82	6.31	7.2	11.9
Phosphorous	2.88	12.1	3.8	29.3	3.33	3.51	5.07	3.15	9.96	9.16	6.7	34.8
pH	6.1	6.7	5.4	6.7	6.4	7.5	7.3	6.1	4.6	4.6	6.5	4.2

Ashton Court

Brandon Hill

Crabtree

The Downs

Oldbury Court

Troopers hill

Supplementary information figure S.2.1. *Layout of the plots within each of the field sites. Satellite* pictures of each of the field sites as indicated at the below of each picture. All pictures were taken from an altitude of 220 m with exception of the picture of Aston court which was taken to an altitude of one km. In each picture the green rectangles represent the plots positioned in each field site.

CHAPTER THREE

THE BOTTOM-UP EFFECTS OF EUTROPHICATION ON FOOD WEBS AND PLANT-FLOWER VISITOR NETWORKS

Paper submitted: Villa-Galaviz, E., S. M. Smart, E. L. Clare, S. E. Ward & J. Memmott. 2019. The bottom-up effects of eutrophication on food webs and plant-pollinator networks. *Proc. Royal Soc. Lond*

Authors' contributions

Jane Memmott conceived the main idea. Edith Villa Galaviz conducted the sampling and analysis. Simon Smart and Susan Ward helped with field logistics and analysis. Elizabeth Claire and Edith Villa Galaviz conducted the bioinformatic analysis.

ABSTRACT

Eutrophication drives non-random species loss resulting in plant communities dominated by grasses with low floral resources. The effects of these changes on other trophic levels, however, are still not well understood. We studied how eutrophication propagates change through the plant assemblage into the plant-flower visitor and plant-herbivore-parasitoid networks using a 27-year old grassland fertiliser addition experiment. Although fertilisers significantly increased the abundance of herbivores and parasitoids, insect species richness was unaffected. In contrast, nectar productivity decreased by 74% and bumble bee abundance by 48%. The loss of forbs species due to eutrophication increased the vulnerability of the flower-visitor and plant-herbivore networks and increased the generality of the parasitoid assemblage. Despite no change in species richness, our results show that the replacement of forbs by grasses due to eutrophication significantly changes the architecture of their associated ecological networks.

INTRODUCTION

Nutrient enrichment or eutrophication is one of the largest causes of plant species decline worldwide (Tilman et al. 2001, Hautier et al. 2015). In grassland, eutrophication leads to the dominance of plant species such as perennial grasses that can capitalise on increased macronutrient supply increasing their biomass production, filling gaps and growing taller, efficiently intercepting light at the expense of shorter or less competitive species (Harpole et al. 2016). The net result is a reduction in diversity where forb species are replaced by grass biomass (Phoenix et al. 2012). Given that grassland has a global extent of 20-40% of land cover (FAO 2015a) and supports a large diversity of culturally valued and functionally important species (Heidenreich 2009) the impact of eutrophication on biodiversity can be considerable.

While eutrophication's impacts on plant diversity are well documented (see Hautier *et al.* 2009, Seabloom *et al.* 2015, Harpole *et al*. 2016) it's effects can also propagate through to higher trophic levels. These changes can be understood as non-random shifts in plant traits of importance for their consumers, for example, decreasing C:N ratio, leading to increased palatability for some herbivores and changes in nectar composition (Throop and Lerdau 2004; Hoover et al. 2012). At the community level, eutrophication decreases pollinator species richness by reducing floral resources (Potts et al. 2010) but increases the abundance (Pöyry et al. 2017) and biomass (de Sassi and Tylianakis 2012) of herbivore species that feed on nitrophilous plants (Smart et al. 2000). In comparison, parasitoids respond to the increase of biomass in the herbivore community by choosing species of bigger body size decoupling species level responses (Stiling and Moon 2005, de Sassi et al. 2012b, de Sassi and Tylianakis 2012).

To date, research on the bottom up effects of eutrophication on other trophic levels has been limited in scope (Tylianakis and Binzer 2014). Very few studies have empirically evaluated the effects of nutrient enrichment using an ecological network approach; and those that have are often short-term experiments (e.g. Burkle & Irwin 2009) or rely on natural gradients rather than an experimental approach (Fonseca et al. 2005). Here I analyse the process of eutrophication using a highly replicated 27-year old experimental grassland manipulation, thereby combining long term data with analytical power. I focus on the effects of eutrophication on two types of ecological networks: plant-herbivore-parasitoid networks and plant-flower visitor networks.

Using this experimental system, I will answer three questions: *Q1: Does eutrophication affect community structure?* Given that eutrophication drives the replacement of forbs by a simpler community of grasses (Stevens et al. 2009), I expect a decline in nectar-producing plants and pollinators, a loss of some herbivore species but an increase in their overall abundance if they

feed on grasses (Stiling and Moon 2005), the latter leading to a concomitant increase in parasitoid abundance. *Q2: Does eutrophication alter the network structure by increasing the number of interactions per species?* Eutrophication promotes the extinction of rare plants (Suding et al. 2005) which may host specialist herbivores (Fort et al. 2016), hence prevalence of generalist species can potentially generate networks with more links per species and lower trophic complementarity (Tylianakis and Morris 2017) where generalist species are concentrated on fewer host species. *Q3: Does the role of dominant plant type mediate the effect of eutrophication on network structure?* Eutrophication increases dominance by favoured plant species (Van Den Berg et al. 2011), and since changes in plant community composition affect herbivores, parasitoids and pollinators (Carvalheiro et al. 2010, Cirtwill et al. 2018) I predict that the changes that cascade through the network structure are ultimately driven by nutrient availability, but are mediated by competitively superior plant species.

METHODS

The Field experiment: Colt Park meadows

Colt Park meadows is a long-term nutrient and plant biodiversity manipulation experiment located at 300 m altitude in the Ingleborough National Nature Reserve in North Yorkshire, England (54°12'N, 2°21'W). The field trial started in 1990 on permanent grassland dominated by the perennial grass species *Lolium perenne* and *Cynosurus cristatus*, on a shallow brown-earth soil (pH 5.1) over limestone of moderate-high residual fertility (15 mg $P_2O_5 1^{-1}$). The aim of the experiment was to test different management strategies for improving the diversity of grasslands in a working agricultural context (Smith 2005, Smith et al. 2008b). Management in 1990 consisted of N:P: K fertilizer application, autumn and spring grazing, sowing of *Rhinanthus minor* and its subsequent removal from half of the plots for three years, and a 21st July hay cutting date. The N:P: K application, grazing and hay cutting both continue to the present day as ongoing treatments.

The experiment consists of 72 plots, each 2.5 m x 6 m (15 m^2) in size, arranged in three blocks of 24 plots. Blocks were set up on a flat land with 20-50 m between each other in hence all plots were under similar physical conditions (figure 3.1 and figure S.3.1 of the supplementary information). Each of the three blocks is subdivided into three sub-blocks of 8 plots corresponding to three levels of seed addition treatments applied from 2004 to 2008: 1) *Trifolium pratense* seed; 2) *Trifolium pratense* and *Ranunculus bulbosus* seed and 3) no seed addition as a control. In each of these sub-blocks three fertiliser treatments have been applied to two randomly chosen plots: N:P: K fertiliser (20:10:10; hereafter NPK,), farmyard manure (hereafter FYM), N:P: K fertiliser + farmyard manure (hereafter NPK+FYM) and a further two plot is left as a control with no fertiliser.

In total, there are 18 plots per treatment (2 plots per treatment per sub-block x 3 sub-blocks per block x 3 blocks) and a total area of 270 m^2 of grassland per fertiliser treatment (Supplementary information, Figure S.3.1).

The management of the field trial at the time of this experiment consisted of sheep grazing from March to mid-May, application of the fertiliser treatments in May, a grass hay crop taken after 21st of July and sheep grazing for two weeks after the hay and cattle grazing during the early winter. The meadow and experimental blocks are based on a working farm and contribute to their annual cattle and sheep production. All sampling on the plots took place during the summer of 2016.

Vegetation, flower and nectar sampling

Plant abundance/cover was sampled every two years from 1990 until 2014 (Allinson and Natural England 2014) and biomass collected for the period 2013-2014. I used estimates of abundance/cover per species from 2014 and biomass per plant functional type (legumes, grasses and forbs) from 2013-2014 to determine the cumulative effect of the nutrient addition treatments on the plant assemblages present (Supplementary information Figures S.3.2, S.3.3 & S.3.4). In 2016 floral abundance was sampled in each of the 72 plots every 3 weeks, and two rounds of sampling were completed before the hay cut (May 31st-July 15th). For each sample, three transects of 5 m were placed regularly in each plot and all flowers within 15cm of each side of the transect line were counted within 24 hours of surveying for flower visitors. In each sampling round, transects were positioned in a different location to cover the whole plot area by the end of the sampling. Plants were identified in the field while the amount of nectar available under each treatment was calculated adding the amount of nectar produced by all the species recorded in each plot. The amount of nectar produced by each species was established by multiplying the amount of nectar produced by one flower of each of the species by the total number of flowers of the species found in the plot. Values of nectar productivity where taken from Baude *et al.* (2016). Although the quality of pollen could be affected by eutrophication (see introduction), sampling for changes in pollen quantity in the field is challenging and there is still not enough evidence that it can be highly affected by eutrophication (see introduction) a difference to nectar productivity where there is wider evidence, since the main aim of this paper is to test how changes in plant resources affect the structure of the network. Nectar productivity represented a more reliable measure.

Insect sampling

Herbivores and parasitoids: All 72 plots were sampled for leaf miners every three weeks (a total of 2 surveys, one in late May early June the other in late June-early July), using the same transect established for floral counting. In each round, all leaf miners were collected and reared in individual rearing pots until an adult leaf miner or a parasitoid emerged. I selected leaf miners as a study group given that their larval development happens inside the leaf, so there is no chance of error when determining their host plant as it occurs with external feeders (i.e. caterpillar larvae).

Flower visitors: Two surveys of 8 minutes each were undertaken in each plot every three weeks one in June and other in July, these consisted of the collection of any insect seen feeding on the flowers during the survey. Surveys took place between 09:00 and 17:00 hrs and in each survey, insects were collected for later identification with exception of bumblebees, which were caught, identified in the field and released.

Insect identification

Flower visitors and adult herbivores were identified by professional taxonomists (see acknowledgements). Parasitoids were sent to the Canadian Centre for DNA Barcoding (Guelph Canada) following the same methods explained in chapter two in the insect identification section. Just as in the previous chapter, due to cost constraints, approximately 80% of parasitoids were sent for identification (1380 out of 1750 specimens). I excluded 50% of the insects of the most abundant specimens of identical specimens reared from the same host; I assumed these to be identical to the specimens with confirmed identifications.

Q1: Does eutrophication affect community structure?

To test if the addition of fertilisers had driven changes in the community structure, I compared abundance of all insect groups and species richness for each trophic level in the network (plants, flower visitors/herbivores and parasitoids) among treatments. I also considered the abundance of bumble bees (*Bombus* spp.) as a focal group, given that there is particular concern over the decline of this group of pollinators (Goulson et al. 2015). Bumble bees were the only bees observed at the field site and due to the low number of bee species observed per plots ($\bar{x}$=1) I did not compare bumble bee species richness among the plots.

Q2: Does eutrophication alter the network structure by increasing the number of interactions per species?

For each of the networks - plant-flower visitor, plant-herbivore and herbivore-parasitoid - I calculated the quantitative version of vulnerability and generality (Bersier et al. 2002). In this context *vulnerability* is the mean number of insect species per plant species and *generality* is the mean number of plant species per insect; these statistics were calculated separately for herbivores and pollinators. In a eutrophication context where loss of plant species occurs, a rise in vulnerability indicates a dependency of the insect community on fewer plant species while an increase in generality indicates an expansion of insect diet or dominance of generalist species. Both metrics were calculated using package *bipartite* in R version 3.3.3 (Dorman and Gruber 2011; R Development Core Team 2009-2016).

Abundance, species richness and network structure metrics were compared using linear mixed effect models, in which levels of fertiliser and seed addition were considered as fixed effects and block as a random effect. When seed addition treatment had no significant effect on the metric tested, it was removed from the model which was rerun with just the fertiliser treatment as fixed effect. Assumptions of the normality of the residuals was tested by Shapiro test and variance homogeneity of the residuals was tested visually. Abundance, species richness and nectar productivity variables were log(x+1) transformed, with exception of abundance of parasitoids which was compared using a negative binomial distribution and abundance of *Bombus* spp. for which I used a zero-inflated negative binomial distribution.

In some networks, web metrics were transformed to fit a normal distribution as specified in Table 3.1 & 3.2 and in the supplementary material (Table 3.1 & 3.2), post hoc analysis section). I tested for the presence of outliers, and models were run with and without up to one outlier. Most results values did not change between models with and without outliers and I report the values of the model without outlier when it provided a better fit. All models were performed in the package *lme4* (Bates et al. 2015) and *pscl* (Zeileis et al. 2008, Jackman 2015) and *Post hoc* analyses were done by Tukey test in the package *multicomp* (Hothorn et al. 2008) in R version 3.3.3 (R Development Core Team 2009-2016).

Q3: Does the role of dominant plant type mediate the effect of eutrophication on network structure?

We used structural equation modelling (SEM; Grace et al. 2010) to test if there is a causal relationship between the impact of nutrient addition and the increase in vulnerability observed in

the structure of plant-flower visitor and plant-herbivore networks and generality in the herbivore-parasitoid networks (see results). SEM was then used to test for a mediating role played by the plant species favoured by the nutrient addition treatments and forbs species richness. That is, does the impact of eutrophication depend on transmission via non-random changes in the plant community?

Smith et al. (2002) observed that the vegetation of Colt Park was characterized by the abundance of *Cardamine pratensis* L*., Ranunculus repens* L. and perennial grasses. Except for *C. pratensis* L., this dominance continues to the present day. Thus, data on biomass and cover from the most recent plant survey in 2014, showed an increase in biomass of grasses with all fertiliser treatments (Supplementary information, Fig S.3.2) and increase in cover of *Ranunculus repens* and persistence of its congeneric *R. acris* (Supplementary information, Fig.S.3.3). Using data from 2014, I compared the proportional difference in total cover of forbs that was attributable to *Ranunculus* spp. (*R. repens* L. and *R. acris* L.) and I observed an increased from 32% in no fertiliser addition to 50% in the highest fertilisation treatment (Supplementary information, Fig. S.3.4).

We tested for the mediating effect of *Ranunculus* spp. and grass species richness in mediating nutrient addition effects onto the structure of the flower visitor network and plant-herbivore-parasitoid network. *R. repens* L. and *R. acris* L. were present in all the treatments but persisted in the fertilised plots as other forbs declined (Smith et al. 2008b, Supplementary information, Fig. S.3.2). *R. repens* can compete with the dominant perennial grasses as it is a vigorous clonal perennial avoided by grazing livestock because of the toxicity of their above-ground parts (Harper 1957a, b). It is also a species tolerant of nutrient enrichment (Stevens et al. 2016). Although, *R. acris* is not as good competitor as *R. repens*, given their very similar flower morphology and nectar production (Baude et al. 2016) I combined the two taxa as I considered it unlikely that pollinators differentiated between them. Similarly, perennial grasses were considered collectively given that, as a functional group, they have increased in abundance and productivity in response to fertiliser addition (Supplementary information, Fig. 3.2). Since leaf miners feed on both forbs and herbivores, and fertilisation mainly favoured grasses (Smith et al. 2008b). For the analysis of the plant-herbivore-parasitoid network, I also included grass species richness as a predictor of vulnerability in addition to forb species richness.

For both *Ranunculus* spp. and grasses, I measure their role in the network by calculating their degree (e.g. Pocock et al. 2011). This metric enumerates the number of links the species supports and therefore conveys the generalism/specialism of a species within a food web (e.g. Emer et al. 2016). Although, normalized degree is better for comparing webs of different size,

given that the calculation of both vulnerability and normalized degree consider the number of insect species, I used degree instead to avoid correlation between these two variables. To facilitate model fitting, abundance, species richness, generality and degree of grasses were log(x+1) transformed while degree of *Ranunculus* spp. was square-root transformed. Analyses were done using the PIECEWISESEM package in R version 3.3.3 (Lefcheck, 2016; R Development Core Team 2009-2016).

RESULTS

In total 191 species of plant and insects were found in the 72 plots: 34 species of plant (29 of which were forbs and the remainder grasses) 93 species of pollinator (n=953), 32 species of leaf miner (n=4614) and 32 species of parasitoids (n=1750). The overall network is shown in Figure 3.2 and a species list is provided in the appendix 2, Table 1-4. All results are based on models where seed addition was removed due its lack of significant explanatory power.

Q1: Does eutrophication affect community structure?

Species richness: Insect species richness was unaffected by any of the fertiliser treatment; thus, there was no significant difference in flower visitor, herbivore or parasitoid species richness among the fertiliser treatments when looking at all the data together (Table 3.1). However, plant species richness in the flower visitor network was significantly lower in the NPK+FYM fertilisers (vs. control, z= -3.767, d.f.=3, p=<0.001) with no significant difference between the rest of the fertiliser treatments and control. Moreover, while herbivore species richness was unaffected by any of the fertilizer treatment, the number of herbivore species attacked by parasitoid was significantly higher with the addition of NPK and NPK + FYM fertilisers (Table 3.2).

Species abundance: All the fertilisers treatments significantly increased abundance of herbivores, on average by 50% (X^2=23.71, d.f.=3, p=<0.0001), and the abundance of parasitoids by 60% (X^2=19.58, d.f.=3, p=0.0002). The most abundant leaf miners in the study, are generalist species of grasses and buttercups, while rare species are specialised to other forbs. The increase in abundance of leaf miners and parasitoids was mainly due to an increase in the leaf miners feeding on *Ranunculus* spp. (Fig. 3.3). *Phytomyza ranunculi* Schrank in particular increased, along with a common parasitoid attacking agromyzid leaf miners, *Dacnusa laevipectus* Thomson. While the collective abundance of the flower visitors was unaffected by the addition of fertilisers (X^2=1.92, d.f.=3, p=0.5881), when considered separately the abundance of bumble bees declined by 48% when NPK+FYM was added (vs. control, z= -2.963, d.f.=3, p=0.0161, Fig. 3.4b).

Nectar plant richness and nectar productivity: NPK + FYM significantly decreased the species richness of nectar producing plants, i.e. the forbs (vs. control z=-3.11, d.f.=3, p=<0.001).

Similarly, fertilisers significantly reduced nectar productivity (X^2=58.89, d.f.=3, p=<0.0001); thus NPK+ FYM and FYM decreased nectar productivity by 74% (z=-7.432, d.f.=3, p=<0.0001) and by 40% (z=-3.232, d.f.=3, p=<0.0001) respectively, while NPK fertiliser only, had no significant effect (z=-1.945, d.f.=3, p=0.3092; fig. 3.4a).

Q2: Does eutrophication alter the network structure by increasing the number of interactions per species?

In the flower visitor networks (Table 3.2) NPK and NPK + FYM addition significantly increased vulnerability, i.e. the number of insect species recorded per plant species (vs. control z=3.13, d.f.=3, p=0.009; z=2.73, d.f.=3, p=0.03, respectively) with no significant changes in generality, i.e. number of plant species per insect species. I observed the same pattern in herbivore networks; NPK and NPK+FYM addition significantly increased vulnerability (X^2=24.31, d.f.=3, p=<0.001) with no effect on generality (Table 3.2). However, in the herbivore-parasitoid networks (Fig. 3.3), the addition of NPK and NPK+FYM increased generality (vs. control, z=2.78, d.f.=3, p=0.03; z=3.56, d.f.=3, p=0.002, respectively) with no effect on vulnerability (Table 3.2); while FYM increased vulnerability (vs. control, z=2.75, d.f.=3, p=0.03) with no effect on generality (Table 3.2). Thus, inorganic and organic fertilisers affect the structure of the food web differently.

Q3: Does the role of dominant plant type mediate the effect of eutrophication on network structure?

Based on the results from the previous question, I analysed the effects of eutrophication on vulnerability as this was the only web metric consistently affected with fertilisation (Table 3.2). For the plant-herbivore network analysis, I also tested if increase in vulnerability in this network led to the observed increase in generality in the herbivore-parasitoid networks. In both flower-visitor networks (Fig. 3.5a) and plant-herbivore-parasitoid networks (Fig. 3.5b), the changes in vulnerability were significantly mediated by a decrease of forbs species richness and an increase of degree of *Ranunculus* spp. (marginal variance R^2=0.57) and grasses (marginal variance; R^2=0.37) both relative to the controls. In the flowers-visitor networks, the SEM also showed that the decrease of forbs species due to eutrophication also increases the importance of *Ranunculus* spp. in the network (marginal variance, R^2=0.25). In the plant-herbivore network, increased vulnerability was caused by the increase of degree of grasses produced by the rise in grasses species richness and loss of forb species richness promoted by eutrophication (marginal variance R^2=0.36). This increase in vulnerability in the herbivore networks, lead to an increase in the generality of parasitoid networks (marginal variance R^2=0.15).

DISCUSSION

Our results show that eutrophication affects the structure of insect networks even when insect species richness remained unchanged. Fertilisers increased herbivore abundance by an average of 50% and parasitoids abundance by an average of 60%, and NPK+FYM decreased nectar productivity by 74% and bumble bee abundance by 48%. Eutrophication also changed the architecture of the networks as it increased the vulnerability of flower visitor and herbivore network indicating a concentration of the insect assemblage onto fewer plant resources. In this section, I first consider the limitations of our approach and then consider our results in the context of the wider literature.

Limitations

There are three main limitations to our research. 1) Although the study was done in a long-term experiment, the data was collected only for one year, so I was unable to detect temporal variations (Rafferty and Ives 2011). 2) The size of the plots was small, so the results reported correspond to behavioural responses for the pollinators; that said Orford et al. 2016 demonstrated that small plots predict farm scale responses for this group. 3) Plots were close to each other which allowed for insect mobility across the experiment. Despite these limitations our main results broadly match the effects of eutrophication on grasslands reported in literature: loss of forbs species and dominance of nutrient competitive species (Phoenix et al. 2012, Harpole et al. 2016). Here though I also show that these two changes affect the structure of insect food webs. This plant mediated effect with no change in alpha diversity was also observed by de Sassi et al. (2012a) when considering just the herbivores and parasitoid communities in a nitrogen enrichment experiment.

The effect of fertilizers on community structure

Fertiliser addition decreased nectar resources and increased the abundance of herbivores. Unlike other experimental studies (de Sassi and Tylianakis 2012), but similar to those from natural gradients (Fonseca et al. 2005) and single plant studies (Moon and Stiling 2000), I found that the abundance of parasitoids also increased with eutrophication, most probably as a response to the increase in herbivores. However, I did not observe a decline in species richness of any of the insect groups, nor did I see a decline in the abundance of pollinators collectively, although I did in bumblebees.

Smith et al. (2008b) showed that the plant community structure in our plots had changed; grass dominance increased and overall, species richness declined with nutrient addition. The decline in species richness, match with the observed loss of nectar productivity and decrease of bumble bees in the NPK + FYM treatment, this being the treatment with the lowest forb species

richness and nectar productivity. Unexpectedly, nectar productivity was not significant different between NPK and control. It is possible that the existing species of grasses, in the experiment, are not high-yielding responsive varieties, able to exploit luxury levels of NPK. This would reduce the ability of NPK to drive a competitive suppression of forbs (nectar plants) via fast-growing competitive grasses.

Even though, the abundance of herbivores and parasitoids rose, species richness remained unaffected. However, the increase in herbivore abundance was mainly due to herbivores of the nutrient resistant *Ranunculus* forb genus, with no change in their herbivore species richness (Fig. 3.3) This lack of change in species richness has been also observed in gradients of agricultural intensification where changes in vegetation leads to increase in the abundance of some species without impacting on species richness (Tylianakis et al. 2007). This leads to the conclusion that functional homogenization across food webs in response to more intensive human activity can arise independently of changes in alpha diversity but solely because of changes in relative abundance and the concentration of interactions on fewer species (cf. Smart et al. 2006) .

Despite the web metrics responding differently to fertilisers for the three insect guilds, I observed that at least one fertiliser treatment increased vulnerability in all networks (NPK and NPK+FYM for herbivores and flower visitors and FYM for parasitoids). This result indicates the dominance of few generalist species and loss of complexity following the addition of fertiliser. However, I only found significant differences in generality in the parasitoid networks. Most parasitoids feed on several polyphagous herbivore (Rodríguez and Hawkins 2000) so, they can flexibly expand their diet whereas many herbivores are much more constrained in their diet (Strong et al. 1984). In the case of pollinators, although this type of interaction also tends to generalization (Waser and Ollerton 2006), a reduction in floral resources limited a diet expansion.

Bottom up effects are believed to weaken as trophic level increases particularity by the third level (Scherber et al. 2010, de Sassi and Tylianakis 2012). I however detected changes in generality and vulnerability in our parasitoid networks. When FYM was added, only vulnerability increased in parasitoid networks. FYM is the more common 'traditional' method of maintaining fertility on these grasslands and NPK is associated with a larger loss of plant species diversity in Colt Park (Smith et al. 2008b). Thus, the combination of both artificial fertilizer and FYM are particularly potent in driving change in the plant assemblage and food web.

Tylianakis and Bazer (2014) noted that changes in preferences of parasitoids due to nutrient enrichment could amplify apparent competition and destabilize herbivore-parasitoid

networks. While I observed that parasitoid abundance increased alongside herbivore abundance, their responses at network level varied depending on the form of nitrogen applied. The strongest eutrophication treatment – the combination of highly available mineral N and P and less labile, slow-release FYM – was most potent at driving non-random change in the plant community with bottom-up effects on food webs.

Based on the observed increase in herbivore species richness in the plant-herbivore network and generality of the network when NPK was added alone or in combination with FYM. Besides the increase in vulnerability with no effect on species richness with the addition of FYM. Our results indicate that eutrophication leads to an increase in the interactions of the parasitoids, albeit via different mechanisms depending on the type of fertiliser. Thus, when NPK or NPK+FYM were added, parasitoids feed on "new" species, this inferred from the observed increase in herbivores species in the herbivore-parasitoid network. But with FYM addition, parasitoids increased their frequency of attack on their hosts. These differences show that in response to nitrogen enrichment, parasitoid communities may not respond in a closely coupled way that compensates for increased herbivory, thereby potentially reducing the pest-control service attributable to parasitoids (Tylianakis et al. 2008).

Our SEM of the plant-herbivore-parasitoid network showed that eutrophication affects forbs and grasses differently regarding their presence in the network; thus, forb species richness declined but grass species richness increased. The most abundant leaf miners in the study, are generalist species of grasses and buttercups, while rare species are specialised on other forbs. Generalist miners with became more abundant with the addition of fertiliser, while the links of the specialists were lost due to the decline in species of forbs (Fig. 3.5). A consequence of eutrophication from increased agricultural fertiliser inputs is therefore a loss of specialists in favour of generalist taxa. This pattern has been observed by studies that have evaluated historical changes in Lepidoptera species distribution and abundance, where both polyphagous species and monophagous species of nitrophilous have increased in frequency (Smart et al. 2000).

Both SEMs also showed that the dominance of competitive species and the decline in forb richness, increased vulnerability and, in the case of herbivore networks, they increased the generality of parasitoids. The effects of nitrogen enrichment on changes in plant composition, herbivore assemblages, biomass of herbivores and parasitoids have been previously documented (de Sassi et al. 2012a, de Sassi and Tylianakis 2012). Here I show that eutrophication also leads to a simplified plant-flower visitor and plant-herbivore-parasitoid network dominated by generalist species in which insects are concentrated onto few plant resources partly triggered by the loss of

forb species. Sensitivity to extinction of less abundant species has been pointed out, both for plants under eutrophication (Sudding et al. 2005) and for plant-pollinator species disturbed by human activities (Aizen et al. 2012). Here I show that the loss of less competitive species simplifies the food webs. Interestingly, simplification occurs without changing species richness. Rather it is the architecture of the interactions among species which is changed. A similar situation was observed by Tylianakis et al. (2007) across a tropical land-use gradient; here as forest was degraded into agricultural habitats, the community (tube nesting bees, their parasitoids and predators) changed in structure, but not in species richness.

CONCLUSIONS

This is the first study that has tested the long-term cumulative impact of eutrophication on an ecological network. Moreover, I looked at multiple networks simultaneously and found all were affected by eutrophication. The mediating role of forbs and grasses in structuring insect networks in response to eutrophication highlights the value of separating species richness from a functional perspective. I used network metrics to show how non-random responses to realistic agricultural intensification pressure can cascade through multiple trophic levels. Further, research is needed to assess how such consequences of eutrophication on insect network could affected the efficiency of pollination and pest control ecosystem services. Our approach would also help to understand the effect of changes in atmospheric nitrogen deposition, a phenomenon that causes similar changes in plant functional groups but in which there is insufficient knowledge of the causal relationship between increase in N deposition and effects on fauna particularly on high trophic levels (Nijssen et al. 2017).

Table 3.1. *The results of linear mixed effect models testing for between-treatment differences in species richness.* The abundance of parasitoids was compared using negative binomial distribution, the abundance of *Bombus* spp. with zero inflated negative binomial and the remainder of the variables were compared using a normal distribution and log+1 transformation, with exception of nectar productivity for which we used a box cox transformation, λ=-0.2). Significant treatments (p>z) refers to the fertilizer treatment which was significantly different the control and the p-value is that of the Tukey test.

METRIC	X^2	*d.f*	*p>Chisq*	*Significant treatment (p>z)*
Species richness				
Forb species richness	*47.24*	*3*	***3.086e^{-10}***	***NPK+FYM 0.001***
Herbivore species richness	*2.347*	*3*	*0.504*	*NA*
Parasitoids species richness	*1.833*	*3*	*0.607*	*NA*
Flower visitor species richness	*2.871*	*3*	*0.412*	*NA*
Species abundance				
Abundance of herbivores	*23.71*	*3*	***<0.001***	***FYM <0.001*** ***NPK+FYM <0.001*** ***NPK <0.001***
Abundance of parasitoids	*19.58*	*3*	***0.0002***	***FYM 0.015*** ***NPK+FYM <0.001*** ***NPK <0.001***
Abundance of flower visitors	*1.92*	*3*	*0.588*	*NA*
Abundance of *Bombus* spp.	*10.98*	*3*	***0.012***	***NPK+FYM 0.016***
Nectar species richness & productivity				
Nectar productivity (fertiliser)	*58.58*	*3*	***1.030e^{-12}***	***FYM 0.01*** ***NPK+FYM <0.001***
Nectar productivity (seed addition)	*30.58*	*2*	***2.279 e^{-07}***	***Clover <0.001*** ***Clover + buttercup <0.001***

Table 3. 2. *Results of the linear effect models of the effects of fertilisers on vulnerability and generality of the networks*. The number of species in the first trophic level refers to the number of species of plants in plant-flower visitor networks and plant-herbivore networks, and it refers to the number of herbivores in the herbivore-parasitoid networks. Number of species in the second trophic level refers to the number of species of flower visitors, herbivores and parasitoids. † Log transform for better fit of the model. ‡ Box Cox transformation.

METRIC	**FLOWER VISITORS**			**HERBIVORES**			**PARASITOIDS**		
	X^2	*d.f.*	*p>Chisq*	X^2	*d.f.*	*p>Chisq*	X^2	*d.f.*	*p>Chisq*
Number of Species in the first trophic level	*14.65*	*3*	***0.0021†***	*1.511*	*3*	*0.68†*	*12.91*	*3*	***0.005†***
Number of species second trophic level	*2.871*	*3*	*0.412†*	*2.347*	*3*	*0.504†*	*1.833*	*3*	*0.607†*
Vulnerability	*11.56*	*3*	***0.009***	*20.87*	*3*	***0.0001‡***	*7.64*	*3*	***0.054***
Generality	*3.803*	*3*	*0.283*	*0.975*	*3*	*0.06†*	*14.06*	*3*	***0.003†***

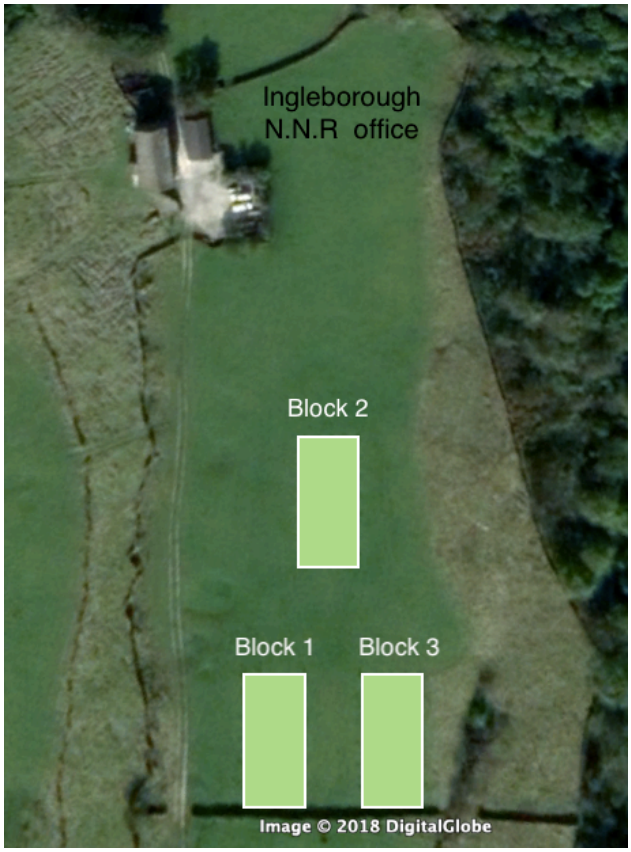


Figure 3.1 *Map of the distribution of the blocks within Colt Park experiment.* Each block is represented as a green rectangle. Ingleborough National Nature Reserve is pointed as reference. The size of the blocks is the real size as seen from an altitude of 656 m**.**

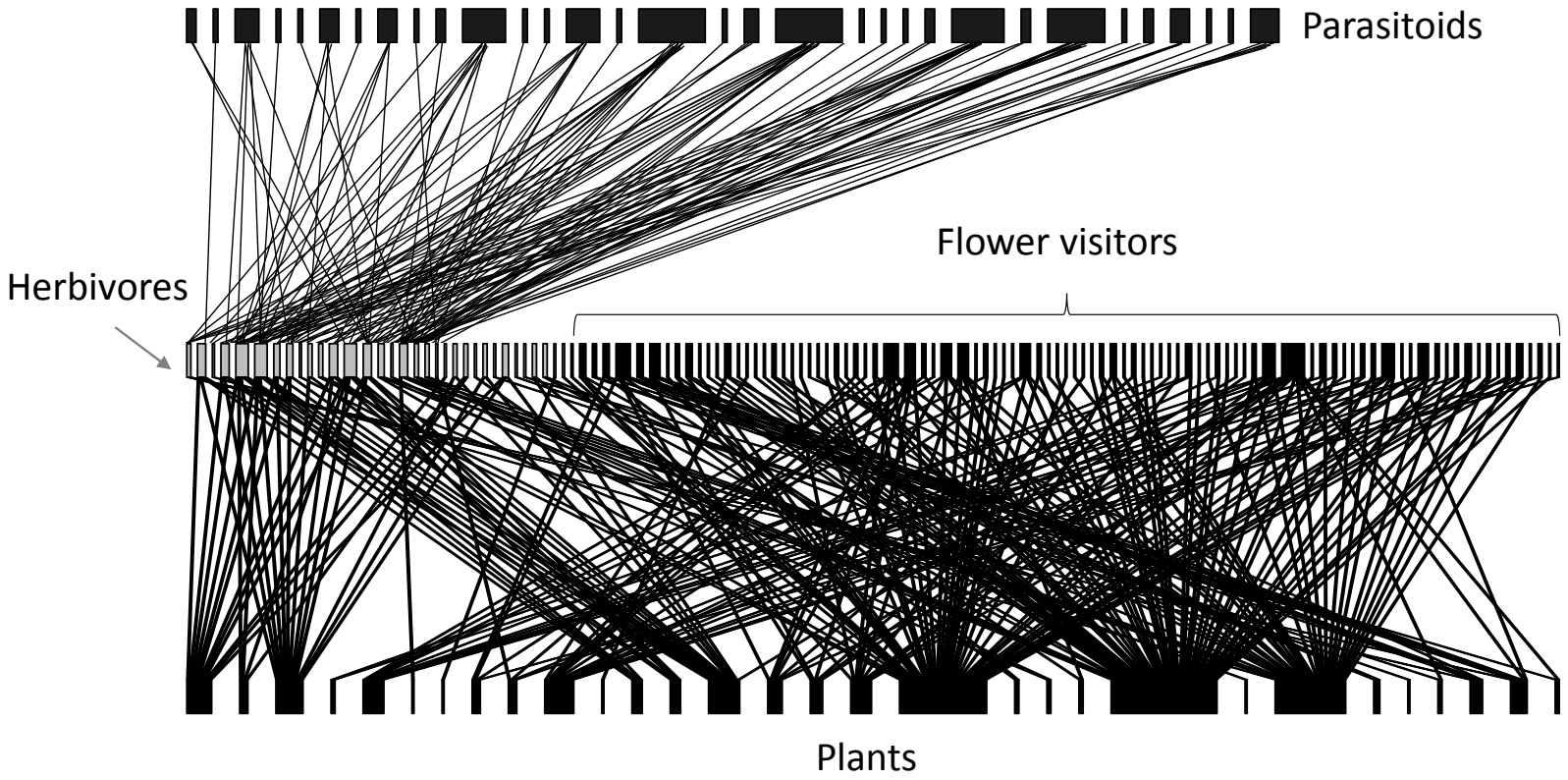


Figure 3.2. *Network of network of all the 191 species sampled in Colt Park experiment.* Each square indicates a species where the size of the squares goes according to the abundance of the species; and lines the interactions between them. First level represents the plant species (39 species), second level in light grey the herbivores (32 and in black the flower visitors (93 species) and third level the parasitoids (32 species).

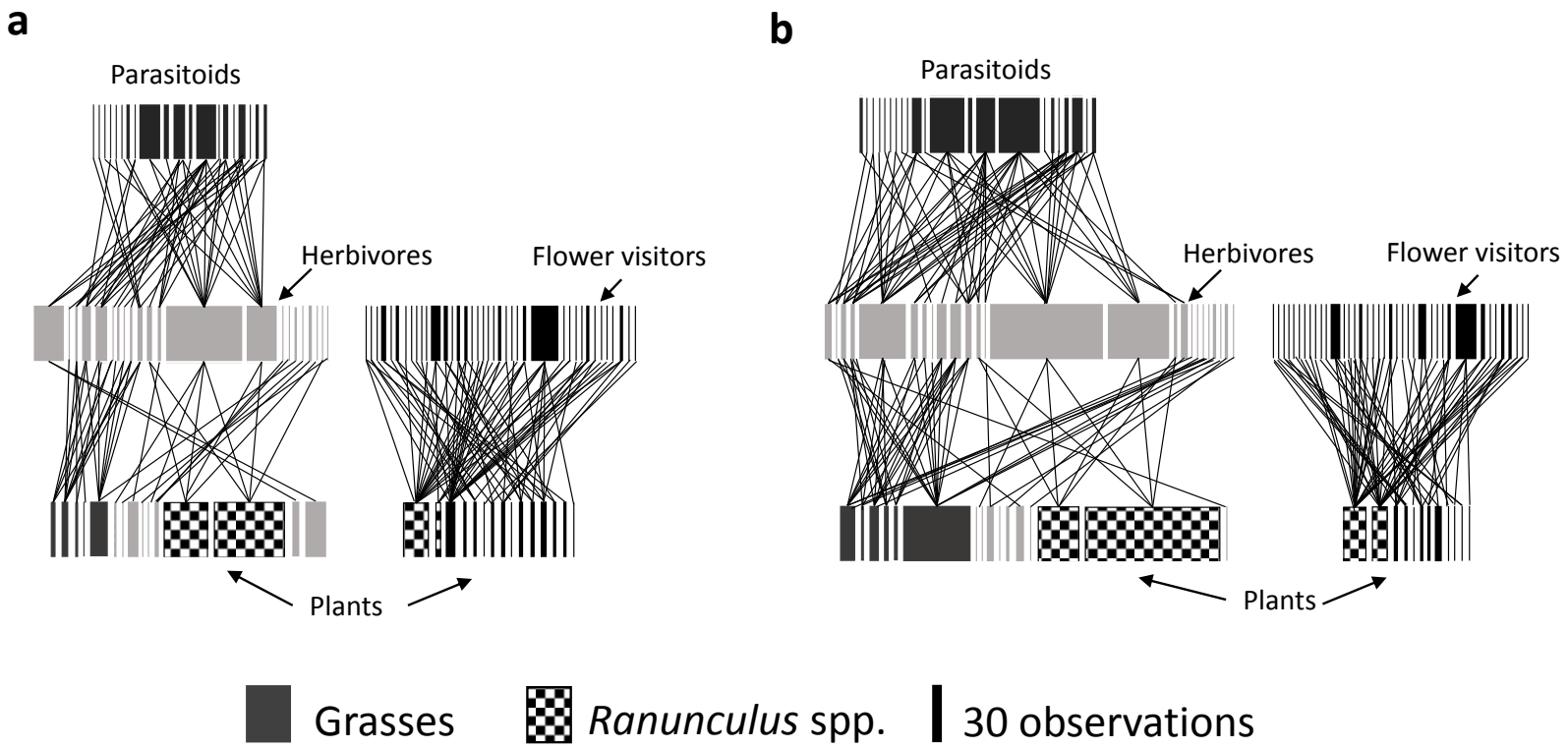


Figure 3.3. *Graphical representation of the sum of all networks of plant-herbivore-parasitoid and plant-flower visitor networks scaled by number of observations in the network data.* a) Control and b) FYM+NPK treatment; Plant-herbivore-parasitoids networks are in light grey with parasitoid species in darker grey and plant-flower visitor in black. Dominant competitive species (grasses and *Ranunculus* spp.) are highlighted as indicated in the figure. The size of the squares represents the abundance of the species where the equivalent of 30 observations is represented as indicated in the figure.

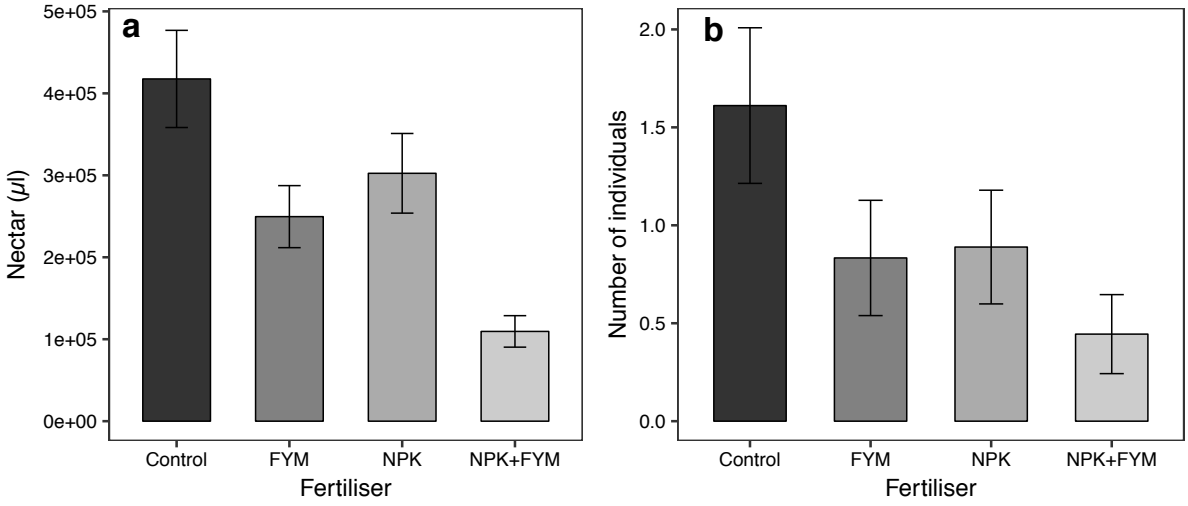


Figure 3.4. *Mean values of a) nectar productivity and b) abundance of Bombus spp. In each of the fertiliser treatments:* control, farm yard manure (FYM), N:P:K (20:10:10; NPK) and farm yard manure plus N:P:K (NPK +FYM). In a) nectar productivity was calculated as the mean of the amount calculated for all plant species in each plot.

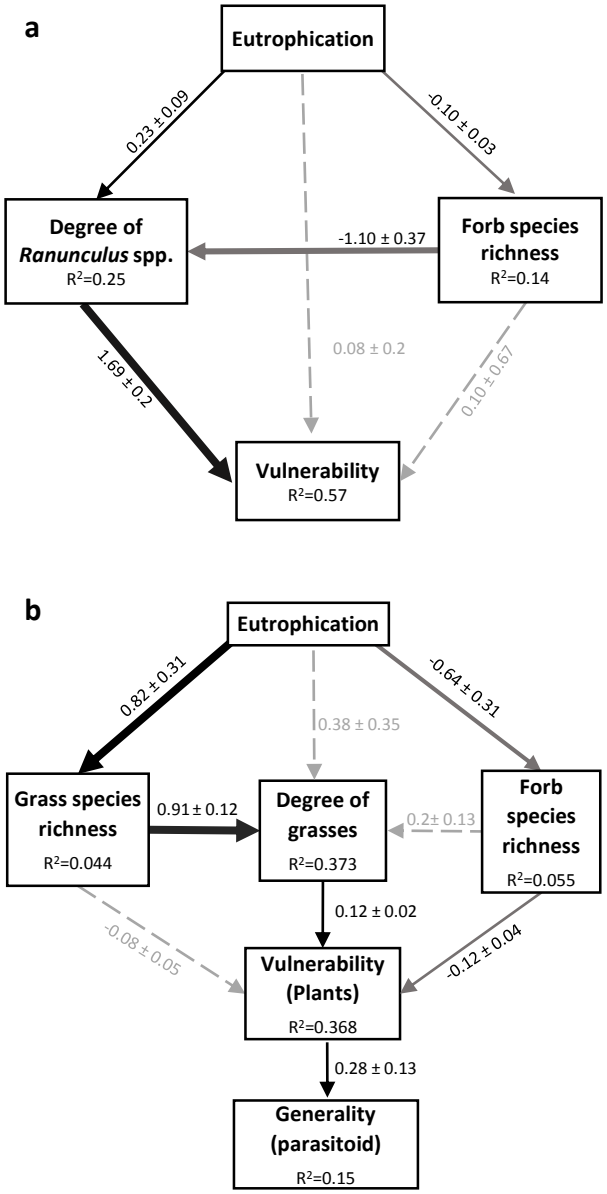
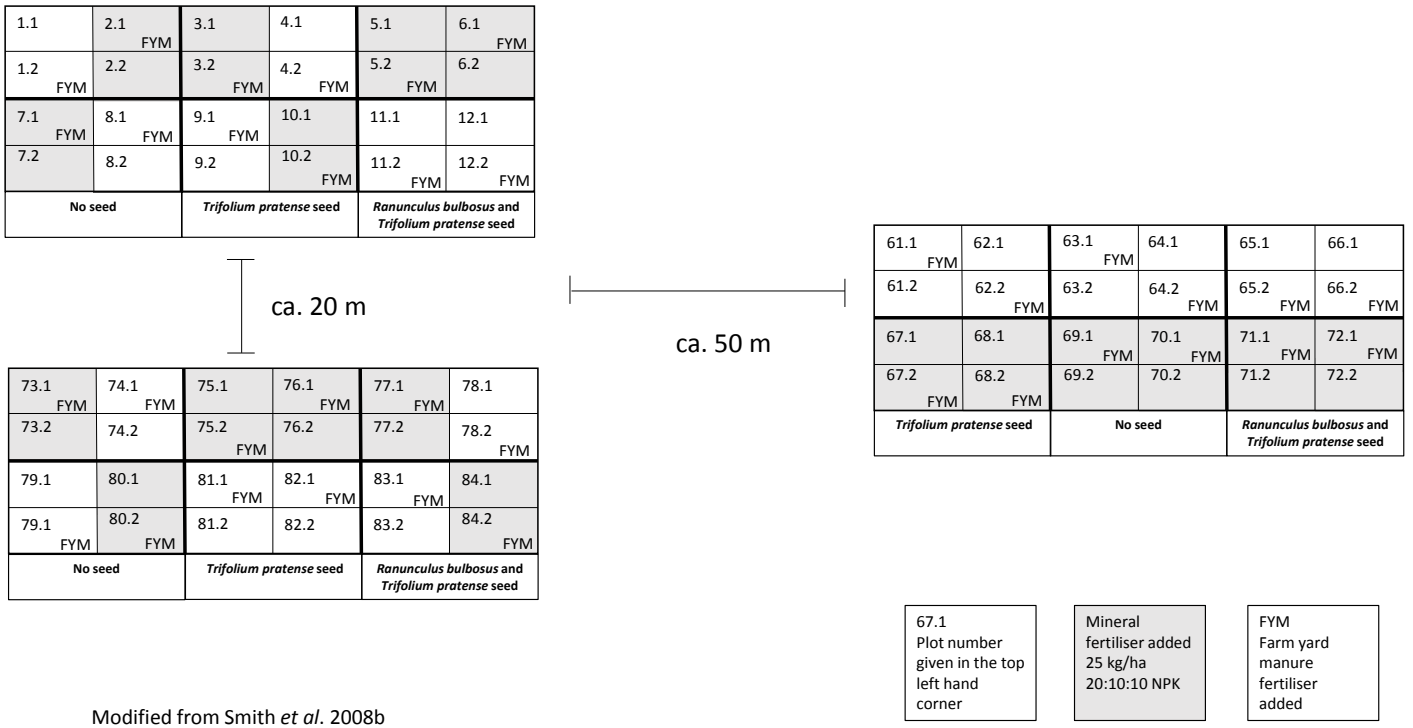


Figure 3.5. *Structural modelling graph of the mediating role of dominant plant type on network structure on:* a) vulnerability of plants in flower visitor network and b) vulnerability of herbivores in plant-herbivore network and generality of parasitoid in herbivore-parasitoid networks. Unstandardized path coefficients are shown with standard error, and the size of the arrow is proportional to the magnitude of the coefficient. Marginal variance of each model is indicated in the box of the response variable of the model. Positive effects are in black and negative in grey. No significant paths are indicated with dashed lines.

SUPPLEMENTARY INFORMATION

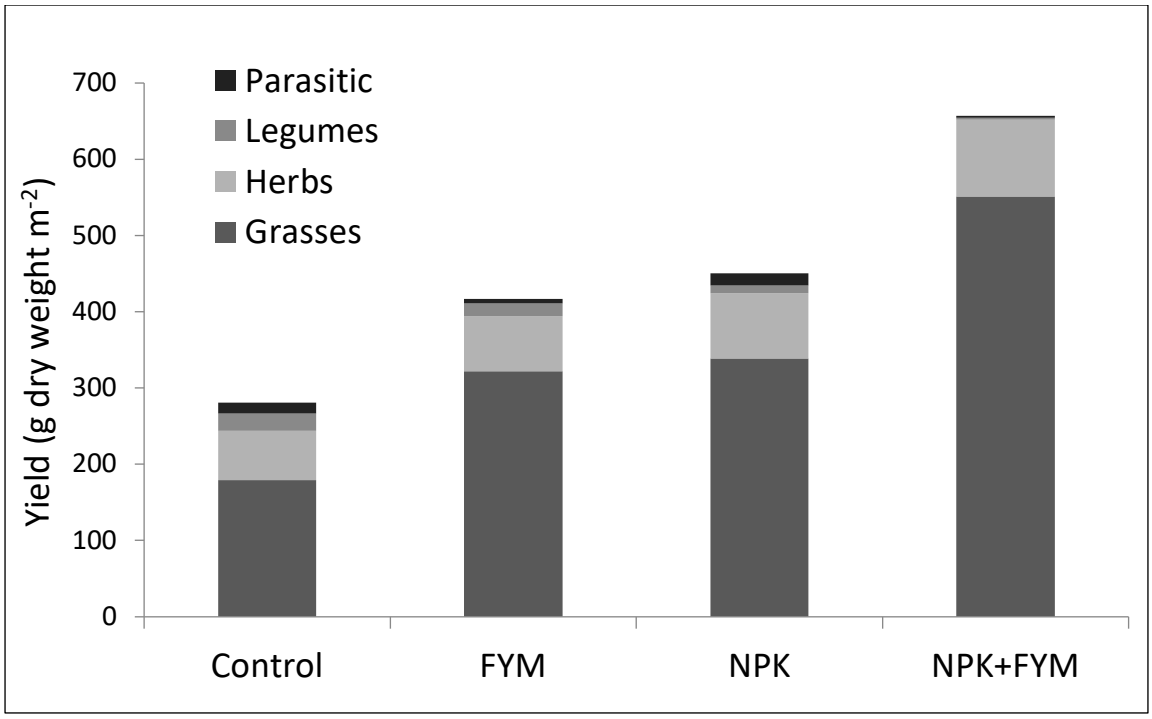


Modified from Smith *et al*. 2008b

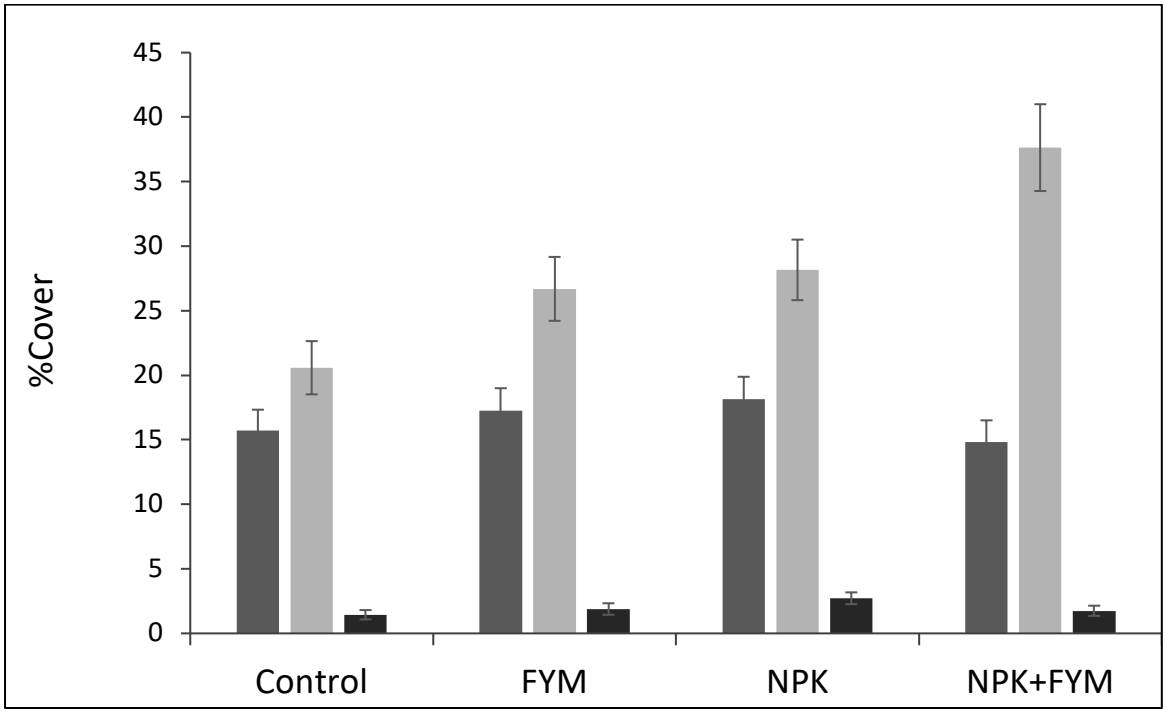
Supplementary information figure S.3.1. *Diagram of the experimental design of the Colt Park Trail as used in this study.* Through the years, Colt Park has been under different experimental phases in addition to the treatments shown in this figure and in the main text. During 1990-1993 some plots were sown with *Ranunculus bulbosus*, in 1999 with *Lotus corniculatus* and *Briza media* and in 2000 *Geranium sylvaticum* was seeded. These treatments were randomly assigned to all the fertiliser treatments. During 2004-2008 Rhinanthus minor was hand removed in half of the plots however, currently however it can be seen growing in all plots

Supplementary information figure S.3. 2. *Photographs of Colt Park experiment.* a) picuture of the plots after the addition of each of the fertilisers treatmets in last May. b) picuture of the meadow before the hay cut, the logs used to limit each of the blocks can be see near the tree line while one of the sticks with a yellow flag that limit each of the plot within each block can be seen at the bottom left of the picture.

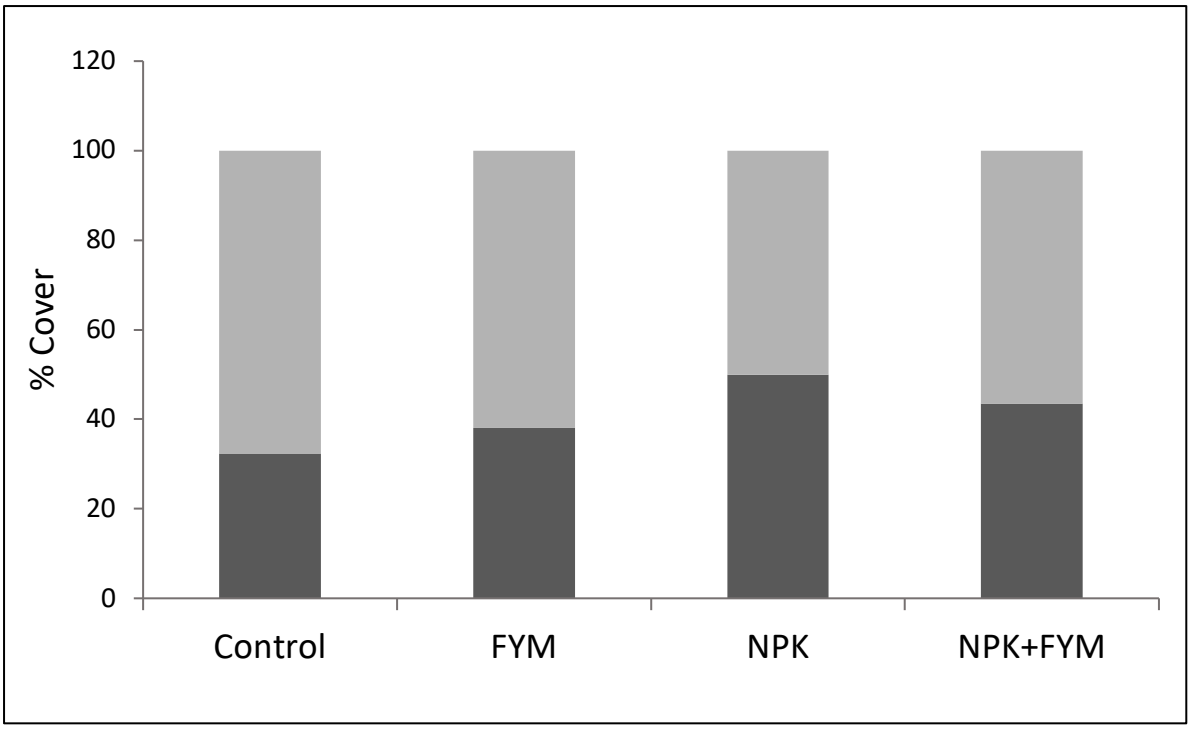
Variations in cover and biomass of dominant species in Colt Park trail



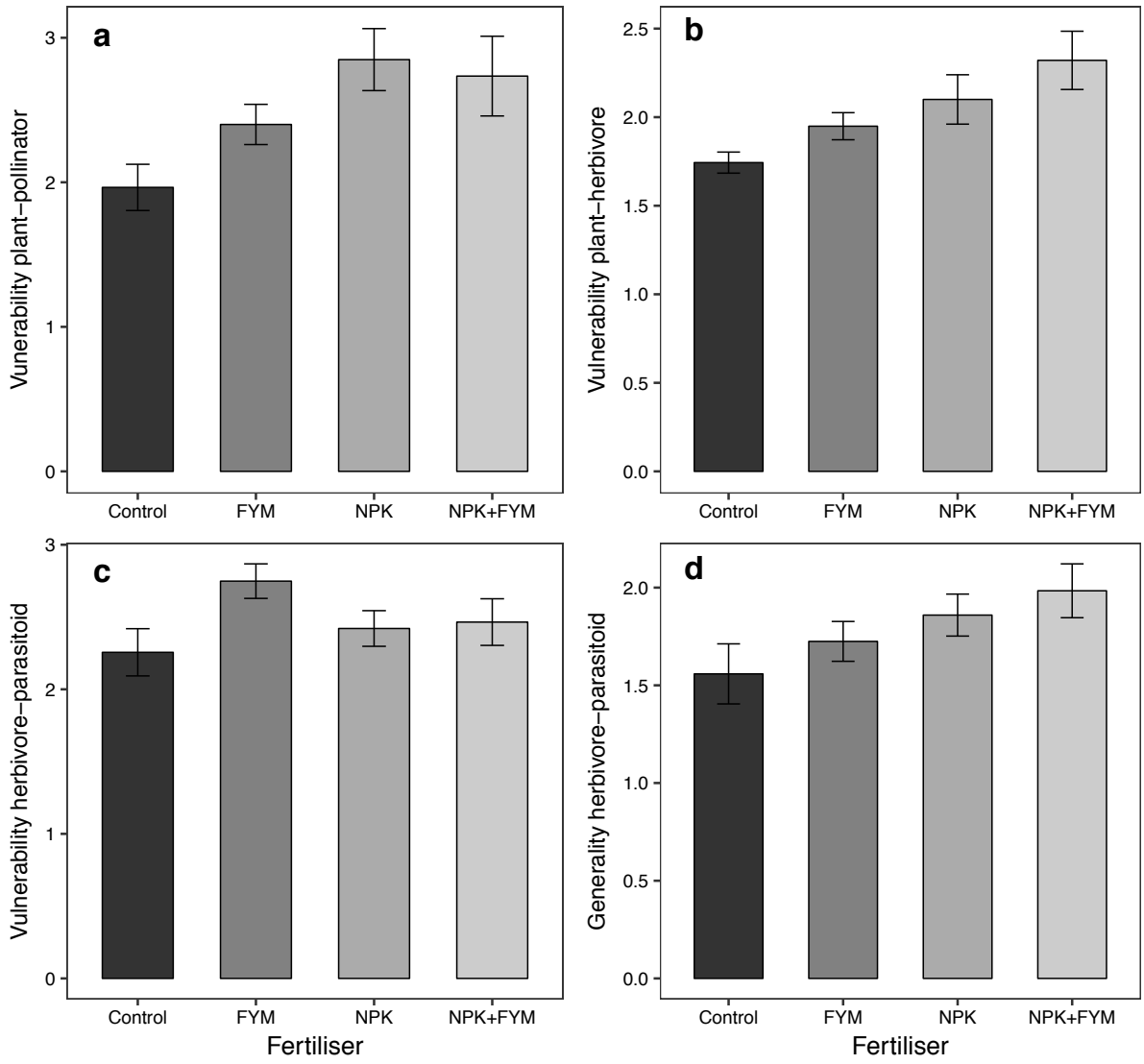
Supplementary information figure S.3.3. *Total Biomass and the contribution of the four functional groups to the total biomass.*



Supplementary information figure S.3.4 Mean percentage of cover of *Ranunculus acris* in dark grey, *Ranunculus repens* in light grey and *Cardamine pratensis* in black. Bars indicate the standard error.



Supplementary information figure S.3.5. *Mean proportion of cover of* Ranunculus *spp. (dark grey) compared to the total cover of non-grasses species* (light grey) in each fertiliser treatment.



Supplementary information figure S.3.6. *Mean values of main metrics affected by eutrophication*: a) Vulnerability of pollinator (mean number of pollinator species per plant species). b) Vulnerability in herbivore networks (mean number of leaf miner species per plant species). c) Vulnerability in parasitoid networks (mean number of parasitoid species per leaf miner species). d) Generality in parasitoid networks (mean number of leaf miner species per parasitoid species. N=72

Post-hoc analysis of the web metrics

Supplementary information table S.3.1. *Community structure* Post Hoc *analysis.* The value of estimate is given ± error standard.

Metric	Treatment	Estimate	z value	Pr(>|z|)
Abundance of herbivores (normal, log+1)	*FYM - control*	0.386 ± 0.08	4.536	**<0.001**
	NPK+FYM - control	0.390 ± 0.08	4.58	**<0.001**
	NPK - control	0.483 ± 0.08	5.668	**<0.001**
	NPK+FYM - FYM	0.004 ± 0.08	0.044	1
	NPK - FYM	0.096 ± 0.08	1.132	0.669
	NPK – NPK+FYM	0.093 ± 0.08	1.088	0.697
Abundance of parasitoids (negative binomial)	*FYM - control*	0.347 ±0.12	2.984	**0.015**
	NPK+FYM - control	0.461 ±0.12	4.006	**<0.001**
	NPK - control	0.435 ±0.12	3.754	**<0.001**
	NPK+FYM - FYM	0.114±0.11	1.044	0.723
	NPK - FYM	0.088 ±0.11	0.796	0.856
	NPK – NPK+FYM	-0.026 ±0.11	-0.242	0.995
Abundance of *Bombus* spp. (zero inflated negative binomial)	*FYM - control*	-0.903±0.41	-2.189	0.126
	NPK+FYM - control	-1.201±0.40	-2.963	**0.016**
	NPK - control	-0.802±0.43	-1.860	0.245
	NPK+FYM - FYM	-0.298±0.28	-1.060	0.714
	NPK - FYM	0.101±0.32	0.314	0.989
	NPK – NPK+FYM	0.398±0.3	1.325	0.547
Forbs species richness (normal, log+1)	*FYM - control*	-0.204±0.084	-2.429	0.077
	NPK+FYM - control	-0.311±0.084	-3.767	**<0.001**
	NPK - control	-0.182±0.084	-2.2	0.123
	NPK+FYM - FYM	-0.107±0.084	-1.283	0.573
	NPK - FYM	0.022±0.084	0.261	0.994
	NPK – NPK+FYM	0.129±0.084	1.567	0.397
Nectar productivity (normal, λ=-0.2)	*FYM - control*	-1.024 ± 0.32	-3.232	**0.01**
	NPK+FYM - control	-2.355 ± 0.32	-7.432	**<0.001**
	NPK - control	-0.655 ± 0.32	-2.068	0.245
	NPK+FYM - FYM	-1.33 ± 0.32	-4.20	**0.0002**
	NPK - FYM	0.369 ± 0.32	1.164	0.816
	NPK – NPK+FYM	1.699 ± 0.3.2	5.364	**<0.001**
	Control-clover	-1.244 ± 0.27	-4.534	**<0.001**
	Control-clover+buttercup	-1.375 ± 0.27	-5.010	**<0.001**

Supplementary information table S.3.2. *Networks* Post Hoc *analysis.* The value of estimate is given ± error standard.

Web	Metric	Treatment	Estimate	z value	Pr(>|z|)
Flower visitor	**Vulnerability (normal)**	*FYM - control*	0.435 ±0.28	1.522	0.424
		NPK+FYM - control	0.770±0.28	2.692	**0.039**
		NPK - control	0.884 ±0.28	3.090	**0.011**
		NPK+FYM - FYM	0.335±0.28	1.170	0.646
		NPK - FYM	0.449±0.28	1.568	0.397
		NPK – NPK+FYM	0.114±0.28	0.398	0.979
Herbivore	**Vulnerability (normal, λ=-1.7)**	*FYM - control*	-0.064 ±0.029	-2.2	0.123
		NPK+FYM - control	-0.130±0.029	-4.46	**<0.001**
		NPK - control	-0.087±0.029	-3.00	**0.014**
		NPK+FYM - FYM	-0.066±0.029	-2.269	0.105
		NPK - FYM	-0.023±0.029	-0.801	0.854
		NPK – NPK+FYM	0.042±0.029	1.468	0.457
Parasitoid	**Herbivore species richness (normal, log+1)**	*FYM - control*	0.097±0.74	1.323	0.548
		NPK+FYM - control	0.194±0.74	2.625	**0.043**
		NPK - control	0.248±0.74	3.314	**0.005**
		NPK+FYM - FYM	0.096±0.74	1.302	0.561
		NPK - FYM	0.150±0.74	2.011	0.184
		NPK – NPK+FYM	0.054±0.74	0.728	0.886
	Vulnerability (normal)	*FYM - control*	0.512±0.18	2.755	**0.029**
		NPK+FYM - control	0.227±0.18	1.222	0.613
		NPK - control	0.255±0.18	1.212	0.619
		NPK+FYM - FYM	-0.285±0.18	-1.533	0.417
		NPK - FYM	-0.287±0.18	-1.543	0.412
		NPK – NPK+FYM	-0.001±0.18	-0.009	1
	Generality (normal, log+1)	*FYM - control*	0.09±0.05	1.848	0.25
		NPK+FYM - control	0.174±0.05	3.563	**0.002**
		NPK - control	0.136±0.05	2.781	**0.027**
		NPK+FYM - FYM	0.084±0.048	1.741	0.302
		NPK - FYM	0.045±0.048	0.947	0.779
		NPK – NPK+FYM	-0.04±0.048	-0.794	0.857

CHAPTER FOUR

VERTEBRATE VERSUS INVERTEBRATE: CAN WE HAVE BOTH IN AGROECOSYSTEMS?

Authors' contributions

Edith Villa Galaviz conceived the main idea and conducted the sampling. Simon Smart and Edith Villa Galaviz conducted the statistical analysis and hay sampling. Susan Ward provided information on hay productivity. Jane Memmott helped with field logistics and writing of the manuscript.

ABSTRACT

Conserving biodiversity in grasslands where practices to increase livestock productivity like fertiliser addition highly affects the diversity of this habitat, has been particularly challenging. Although, there has been interest in maintaining invertebrate species richness in agroecosystems, few studies give equal weight to the fact that farmers need to maintain a profitable level of productivity. This lack of approach makes hard to establish sustainable actions that ensure the preservation of ecosystem services in agroecosystems. In this chapter, I compared the performance of three fertilisers treatments and no fertiliser addition in forage production and enhancement of the nutritional content of forage as well as their impacts on plants, pollinators, herbivore and parasitoid communities in a hay meadow in the North of England. I combined information from previous studies on hay productivity and plant diversity with information I collected myself on insect diversity and hay nutritional content. My results confirmed that the use of organic fertilisers is the practice that offer the closer medium point between insect conservation and livestock production. I also found that the use of inorganic fertilisers does not offer a better performance than farm yard manure. But beyond this, I observed that to achieve an increase of 5% in forage productivity 40% of resources for pollinators are lost with a high impact in the abundance of bumble bees. This shows that enhancing one ecosystem service for economic purposes disproportionately decreases other ecosystem service that could potentially rise the economic cost of food production. Equally, this study highlights the urgent need to work in multidisciplinary groups to establish sustainable management practices in grasslands.

INTRODUCTION

Sustainably increasing food production depends on ensuring that the essential services provided for free by ecosystems are not irreversibly exhausted (Millenium Ecosystem Assessment 2005). Within agricultural practices, intensive livestock production has been particularly harmful for the environment. The negative consequences of different management practices including fertilisation and extremes of grazing intensity, have resulted in widespread loss of habitat and biodiversity as well as contributing to atmospheric nitrogen deposition and climate change (Steinfeld and Wassenaar 2007, Harpole et al. 2016). Regardless of the implementation of mitigation measures, the negative consequences of livestock production are expected to continue (Pelletier and Tyedmers 2010, Herrero and Thornton 2013). This has led to an apparent impasse between conservation and livestock production, where reconciling both their aims remains a remote possibility.

Whilst there is good evidence for the positive effects of biodiversity on livestock production, for example forage from species-rich grasslands is of higher nutrient value than cereals and conventional hay (French 2017), fertiliser input, reseeding and intensive stock management all negatively affect biodiversity. However, profitable livestock production with no large negative impact on wildlife is theoretically achievable (Hessle et al. 2017). In grasslands, rotational grazing can sustain plant diversity and floral visitor populations (Ravetto Enri et al. 2017). It can also increase primary productivity by sustaining interactions for example, between high yielding grass species and nitrogen fixing forbs (Cardinale Bradley et al. 2013, Craven et al. 2016, Oehri et al. 2017). Indeed, grazing for conservation purposes is widespread in the UK and northern Europe (Bignal and McCracken 1996, Primdahl et al. 2003).

While the addition of fertilisers is one of the main causes of degradation in terrestrial and aquatic systems (Smith et al. 1999, Bouwman et al. 2002, Sutton et al. 2013), it is used to accelerate forage production to reach the nutrient levels required for livestock breeding and maintenance (e.g. Richards and Genever 2016; Vickers and Stewart 2016) . However, the use of fertilisers is a major driver of the current pollinator crisis (Chapter 3; Potts et al. 2010; Goulson et al. 2015) . Fertilisation also increases the abundance of insect herbivores (Chapter 3) and thereby risks damage to a fertilised crop as predators may not be able to respond quickly enough (Tylianakis et al. 2008). These types of effects can increase the cost of food production if insect communities are not diverse enough to sustain ecosystem services such as pollination and pest control (Costanza et al. 1997, Losey and Vaughan 2006, Potts et al. 2016). For these reasons, one

of the main aims of wildlife conservation in agroecosystems is to find management practices that maintain beneficial invertebrates alongside livestock production (Ravetto Enri et al. 2017).

Although, there is a considerable amount of research on maintaining invertebrate species richness in agroecosystems (e.g. Andrews & Rebane 1994; Carvell, 2002; Pinches et al. 2013; Orford et al. 2016) , very few of these studies give equal weight to the fact that farmers need to maintain a profitable level of productivity (see Ravetto Enri et al. 2017 for an exception though). This biased approach complicates the acceptance of some of the recommendations for wildlife conservation, since practices like fertiliser application, are necessary to enhance livestock production (Vickers and Stewart 2016) or are part of traditional management regimes (Smith et al. 2008b, Gamble and St. Pierre 2012). In this context, this chapter explores whether there is a fertilisation practice that optimizes both livestock production and insect conservation using a British upland hay meadow as a model system.

I focus on flower visitors and parasitoids as they are providers of key ecosystem services in farmland systems and I ask: *What is the optimum fertiliser practice for both insect and livestock ecosystem services?* Given that the main goal of this chapter is to find a fertiliser practice that provides both insect ecosystem services and livestock production, my aim is to identify the fertiliser regime with the best performance for forage production but with the lowest impact on insect communities.

METHODS

For this chapter I used data from the literature, including the previous chapter, and I collected new data. In total 15 variables were selected based on their impact on insect communities and their benefits to livestock (Table 4.1). I then use randomization tests to determine if there is an optimum fertiliser application practice for these variables, thereby finding an optimum for both biodiversity and livestock production. Note that the optimum is the point that *jointly* maximises levels of both biodiversity and livestock production. The individual maximum values for each variable, independent of the other may be very different and considerably higher than values coinciding with the optimum.

Field site and experimental design

The study was undertaken at the Colt Park hay meadow Field Trial which is based in Ingleborough National Nature Reserve (North Yorkshire, England, grid reference SD775782). Traditional management of hay meadows includes the application of farmyard manure, a waste product of livestock reared under cover during the winter (Gamble and St. Pierre 2012). Although, farmyard

manure supplies high levels of nitrogen, phosphorous and potassium only 20% of these nutrients are available the following year (Smith et al. 2008b). This results in the need for application of artificial fertiliser to realise faster and greater forage production to sustain animals over the winter (Gamble and St. Pierre 2012). Artificial fertilisers are known to have larger negative effects on plant and soil communities than farmyard manure (Andrews and Rebane 1994, Smith et al. 2008b). Nevertheless, their use is widespread since they drive the high grassland productivity required to support demand for dairy, meat and other products in a wide range of global and European farming systems (DEFRA 2016).

Details of the experimental design of Colt Park trial are explained in Chapter 3. In brief though, organic and inorganic fertilisers were applied to replicate plots, both separately and jointly. However, for this chapter, to avoid the confounding effects of other treatments applied within the experiment. I used the 24 plots with no seed addition. This results in six replicates of the following treatments: control plots with no fertiliser treatments, inorganic fertiliser (N: P: K in the ratio 20:10:10; 25 kg ha^{-1} nitrogen plus 12.5 kg ha^{-1} of P_2O_2 and K_2O), organic fertiliser (12 t ha^{-1} of farmyard manure, FYM) and both fertilisers together (NPK+FYM) which is a rather uncommon practice, but it can represent high levels of nutrient addition used in more intensive agroecosystems.

The variables measured

The data consisted of vegetation and hay productivity survey data from 2011-2014 (Allinson and Natural England 2014, Lancaster Environment Centre 2016), pollinator, herbivores and parasitoid abundance and species richness from 2016 (see Chapter 3) and new data on hay quality I sampled in 2017. While these datasets were gathered in different years, they were all collected towards the end of a 28-year experiment and consequently the experimental treatments will have had time to exert a detectable cumulative impact on these variables. Since the 15 metrics each track different aspects of plant, insect communities and forage production, I classified the variables into four groups: insect resources, insect community, livestock resources and livestock production (Table 4.1). The variables in the groups are as follows:

1) *Insect resources*

I included variables that measure the availability of resources for pollinators and herbivores since these influence insect community diversity (Siemann et al. 1998). These variables are: a) plant diversity - an index of the plant community structure- plant diversity is highly related to insect diversity (Siemann 1998, Kemp and Ellis 2017); b) forb species richness - these species are a

vulnerable group with respect to nutrient enrichment (Phoenix et al. 2012 and Chapter 3) and c) nectar production - a measure of energy available for pollinators (Potts et al. 2010, Baude et al. 2016).

Nectar productivity and forb species richness were both collected using the methods explained in Chapter 3 (Methods vegetation, flower and nectar sampling). To measure plant diversity, I calculated the natural logarithm (ln) version of Shannon-Wiener index (Magurran 2004). I used data from the last vegetation survey performed in Colt Park in 2014. This vegetation survey was done by recording species cover within a $2m^2$ quadrat and was carried out in early-mid-June 2014. It consisted of recording the number of times each species touched one of the 100 pins of a quadrant located in the centre of each plot (Allinson and Natural England 2014).

2) Insect communities

For this group, I included variables that describe the structure of insect communities and so could indicate changes in the provision of insect ecosystems services. This combines information on the diversity of: a) flower visitors, b) herbivores and c) parasitoids, under the assumption that conserving biodiversity enables ecosystem services (Balvanera et al. 2006, Macfadyen et al. 2012, Garratt et al. 2016); d) abundance of bumble bees this being an indicator of the effects of fertilisers on a group of conservation concern which is negatively affected by eutrophication (Chapter 3, Goulson et al. 2015, Stevens et al. 2018) and e) percent parasitism, as a measure of pest control, since diversity of parasitoids is not related to efficiency of pest control (Rodríguez and Hawkins 2000). Percent parasitism was calculated by dividing the number of parasitoids emerged by the total number of insect emergent.

Data on flower visitors, herbivores and parasitoids were collected in 2016 as explained in Chapter 3. However, this dataset now includes an extra round of sampling for flower visitors that was made only in the no-seed-addition section of the experiment and so not included in chapter 3. The following new variables were calculated for each treatment: percent parasitism, diversity of pollinators, diversity of herbivores and diversity of parasitoids. The diversity of each group was calculated using the ln Shannon-Wiener index (Magurran 2004). All diversity calculations were carried out using the R program *vegan* (Oksanen et al. 2017) in R package version 3.3.3.

3) Livestock resources

Variables for this group consist of common measures to evaluate the forage productivity and forage quality on farms (Gammon 2012) and are as follows: a) mean dry weight of the hay for the period 2011-2014, b) ruminant metabolizable energy in the hay - energy that is available to the

cow after accounting for losses in digestion, gases and urine; c) hay crude protein or nitrogen content, d) hay ash - a measure of the dry matter that is mineral rather than organic, e) total fat content of the hay (technically known as oil B), and f) Neutral cellulase gammanase digestibility of the hay - this measures the digestibility or fibre content of the hay (a summary of the methods used by the laboratory, is included in the calculation of the hay quality variables in the Supplementary Information).

Forage productivity was measured as the dry mean weight of the hay during the period 2011-2014 using data collected by Lancaster Environment Centre (2016) . For this, hay was cut at peak biomass just before harvest from the centre of each plot (1m from the plot edge) and dried in an oven at 60 °C for 48 hours and weighed (see Lancaster Environment Centre 2016) . Annual records of forage productivity have showed that in despite of the temporal variations in dry weight the differences between fertiliser treatments keep constant through the time. Addition of both FYM and NPK is the treatment that increases hay productivity while no fertiliser addition provides the lowest productivity. In this sense calculating the mean value is a representative measure of the effects of the fertiliser treatments through time. Forage quality data was collected by myself in July 2017 one week before the annual hay cut. The samples were collected by placing three transects of 5m separated by 50 cm in each plot, and a sample of c. 20 g of above-ground biomass was clipped to ground level once a meter along each transect. All the samples were combined to provide a total of 200 g of hay per plot. Each sample was then oven dried for 48 hours at 65°C and analysed for the variables listed in the previous paragraph by Sciantec Analytical services (http://www.sciantec.uk.com/services.php?service=forage).

For all 15 variables, with exception of ash content in forage, high levels mean high positive impacts. Content of ash in forage higher than 13% can cause soil contamination and secondary fermentation during hay digestion (Gammon 2012). In order to be able to meaningfully compare this value with the others (i.e. to maintain the “high level equals a positive effect” interpretation). I calculated the difference from the actual value of ash content and the maximum recommended (13%) and subtracted the value from one. For example, 5% becomes 8% and 8.1% becomes 4.9% maintaining the “high level equals a positive effect”.

4) Livestock production

To estimate the livestock production that each treatment could support. I calculated the number of suckler cows (600kg) that could be fed overwinter, with the hay produced by one hectare under each treatment. To calculate a measure in kilograms per hectare, I first transformed the mean grams per meter of hay produced and scaled up to one hectare. Then, I calculated the amount of hay that could

be consumed in a day by the cattle. This was done to ensure that the hay produced lasted the standard 24-week period that the cattle are reared indoors during the winter (Backshall et al. 2001). The latter was estimated by dividing the amount of hay produced in one hectare by the total of days the cattle are indoors (168 days). Finally, the kilograms of hay available every day was divided by 7.5 kg, which is the mean amount of hay that a suckler cow of 600kg needs per day for maintenance (Gammon 2012). I chose suckler cows because in the upland grasslands in England, cattle are mainly bred for meat production and breeding stock (Chesterton 2009).

Calculating the optimum fertiliser practice for both insect and livestock ecosystem services

First, all 15 variables were compared individually, then to have a broader comparison that allows to picture a win-win scenario. I used a second approach in which I compared the four groups of variables: insect resources, insect communities, livestock resources and livestock productivity. However, given a correlation between some variables, the number of variables was reduced from 15 to 9 and the number of groups from four to three (insect resources, insect communities and livestock resources, i.e. dropping livestock productivity). Both approaches were kept as the 15 variables convey greater detail regarding the attributes of the ecosystem that contribute to the delivery of ecosystem services.

Variables analysed individually

I used randomization tests to identify the optimum fertiliser practice across the variables measured. Randomization test also known as permutation tests or Monte Carlo permutations are powerful statistical tests that do not rely on parametric assumptions regarding the distribution of the data. They are especially useful for complex designs and low numbers of samples that reduce the statistical power of parametric tests (Hooton 1991, Nuzzo 2017). Since randomization tests involve the creation of a probability distribution built with the pool of samples for all treatments to which the value of interest is compared, they offer a direct evaluation of how well or how badly the performance of one treatment is in comparison of the performance of all treatments, which is the aim of this chapter.

I compared the rank of the observed mean of each variable to the rank of the mean of a random sample from the pool of samples. This comparison was done by performing the randomization test as follows: 1) I ranked the mean values of each fertiliser treatment for each of the variables studied; values were ranked in a scale of 4 to 1 where 4 was assigned to the treatment with the highest performance and one to the treatment with the lowest performance, 2) I randomly selected 6 values (the same number as the number of replicates in the experiment)

from the pool of plot values. I calculate the mean and rank the value following the same criteria used for the observed means; and I repeated the process 10,000 times to build a probability distribution; 3) I calculated the probability that the observed mean rank of the treatment tested was higher than the mean rank calculated from the random samples created with the randomization. This was done by counting number of times out of the 10,000 randomizations the observed mean of interest was ranked higher than the rank means of the random sample taken from across the fertiliser treatments. All steps of the randomizations including the correlation tests were done using packages *vegan* (Oksanen et al. 2017), *dtplyr* (Wickham 2017)*, plyr* (Wickham 2011), and *ggplot2* (Wickham 2009) packages of R version 3.3.3 (R Development Core Team 2009-2017).

Variables analysed by the four groups

As explained before, to facilitate the understanding of the pros and cons of each fertiliser and to try to identify a win-win scenario, I compared each group of variables by combining the values of the variables within each group. Because variables are measured in different units, I standardised the values by subtracting the mean of each variable within each group and dividing it by its standard deviation. Then, to test if there was a fertiliser treatment that offers a win-win situation within treatments. I calculated the mean value across all groups. This mean represents the average level of support across all group indicator variables. Then, to compare the response of the cross-group mean between fertiliser treatments, I performed the same randomization test as described above.

However, to fairly compare between groups (*i.e*. insect communities vs livestock resources) groups must have an equal number of variables. Differences in the number of variables per group can been solved by two approaches: by calculating a weighted mean (weighting by the number of variables in each group) or by removing some variables until all groups had an equal number of variables. Because correlation between the hay quality measures is expected, which I confirmed by doing a correlation Pearson test (Table 4.2), I chose removal of variables as the best approach. I reduced the number of variables from each group achieving an equal number of three variables in each, three variables being the lowest number of variables remaining in a group after checking for correlations. A further consideration is the fact that livestock productivity group is entirely based on forage productivity and so is not an independent variable and was removed from the analysis. As a result, a comparison could be made between three groups: insect communities, insect resources and livestock resources. For which a single variable was created by

combining the values of three selected variables for each group. The selection process was as follows:

For insect resources the group with the lowest number of variables (n=3), all the variables were kept: forb species richness, nectar productivity and plant diversity. In the case of insect communities (n=5 variables), since all variables offered information on how insect communities respond to fertilisation (chapter 3), the elimination of one of the variables would mean losing information on one insect community. Therefore, the most parsimonious action was to combine all the information for species richness and abundance for herbivores, parasitoids and pollinators in a single variable named insect diversity (ln Shannon-Wiener, Magurran 2004) . In this way the 5 variables of this service were reduced to the following three: parasitism rates, insect diversity and abundance of bumble bees.

For livestock resources, the group with the greatest number of variables (n=5), I did a Pearson correlation test between all the variables. This analysis showed a correlation between crude protein and the rest of the of the hay quality measures but no correlation between mean dry weight and hay quality measures (Table 4.2) indicating independence of hay quality from hay productivity. Strictly, this procedure reduced the number of variables to two (mean dry weight of hay and crude protein), however I decided to also include ruminant metabolizable energy due the importance of energy as indicator of nutritional quality (Gammon 2012). This measure combines information on oil and protein content and therefore there is a correlation with these two variables. In livestock production metabolizable energy is an important limiting factor and an essential measure to evaluate the suitability of the forage for the type of cattle (Vickers and Stewart 2016).

RESULTS

Variables analysed individually

The control treatment was the treatment with the lowest mean for livestock resources, especially for crude protein, moisture, Oil b and mean dry weight while NPK+FYM was the lowest for insect resources, abundance of bumble bees and diversity of pollinators. NPK, was the lowest for: ruminant metabolizable energy and neutral cellulose gammanase digestibility, and although it was not ranked as the lowest for any of the variables of insect resources it was the lowest for parasitism rates and diversity of herbivores. Meanwhile, FYM only ranked as the lowest within the fertiliser treatments for diversity of parasitoids (Table 4.3 &4.4).

When comparing, the number of animals that can be fed with each of the fertiliser treatments, the control was the worst and NPK+FYM was the best (Fig. 4.1c &d) with a difference of 1.7 or 2 animals between the two treatments. Within the three fertilisers treatments, NPK was the least productive. However, differences between fertiliser treatments amounted to less than one cow per hectare, showing than in terms of livestock production there is a small difference between treatments.

Variables analysed by the three groups

When compared by group, the differences between fertilisers treatments detected when each variable was measured individually were more evident. The control was the worst for livestock production but the best for insect resources and insect communities. NPK+FYM was the worst for insect resources and when all groups of variables were measured together. However, it was neither the best for livestock resources (p=0.8) being the second behind FYM (p=0.9). NPK was the worst for impacts on insect communities. Finally, although FYM was the second worst for insect resources it was optimal for values across all groups (Fig. 4.2 & table 4.4).

What is the optimum fertiliser practice for having both insect conservation and livestock production?

In summary, while no fertilization is inadequate for meat production in upland grasslands (Fig.4. 2), the addition of artificial fertiliser reduces pest control when applied in isolation and decreases nectar productivity when applied in combination with FYM (Fig. 4.3). In this sense, FYM was the closest treatment to a win-win scenario, being the second-best for livestock resources and livestock productivity with medium negative effect on insect communities and insect resources. The dividend realised from combined application of NPK+FYM on livestock resources, relied mainly on productivity and crude protein which are the main reasons farmers apply mineral fertilisers (Vickers and Stewart 2016). However, NPK+FYM application compared to FYM, increased the hay productivity by just 5% and crude protein by 0.5% but decreased nectar productivity by 40%.

DISCUSSION

There was no fertiliser treatment that had the best performance for all the four groups studied here, showing that achieving a perfect win-win scenario in agroecosystems is not (as expected) straightforward to achieve. Thus, the control (i.e. no fertiliser) was the best treatment for insect conservation but the worst for livestock resources, while the addition of both NPK and farm yard manure combined was the worst for insect conservation but the best for forage production,

though not for forage quality. The addition of NPK alone did not produce higher hay productivity or hay quality than FYM, but it had a higher impact on the insect communities, mainly via a reduction in percent parasitism, although it was better for insect resources. FYM was the fertiliser practice that offered the closest to a win-win situation being better than NPK for hay productivity and hay quality, and better for insect conservation when applied alone than when applied in with NPK. An advantage of the approach I used is that the magnitude of the advantages and disadvantages of each treatment can be compared. For example, the fact that in comparison to FYM alone, the combined NPK+FYM treatment had only a marginal effect on hay productivity (5%) and hay quality but an environmental cost of a 40% decrease in nectar productivity. In what follows, I first present the limitations of my work and then put my results in the context of the wider literature.

Limitations

There are four main limitations to this study. First, the amount of fertiliser applied in the Colt Park experiment was applied according to the maximum permitted by the environmental stewardship scheme (12 tonnes per hectare for FYM from the 18 tonnes permitted and the limit of 25 kg/ha of inorganic nitrogen fertiliser) - but the amount of fertiliser used outside of stewardship is far greater, particularly in permanent grassland where the addition of inorganic fertilisers is an average 91 kg/ha (DEFRA 2018a). However, the amount of FYM and NPK are representative of the fertiliser doses applied in upland grasslands. A higher application rate could improve the performance of mineral fertiliser for livestock production but likely with greater impacts on ecosystem services. As shown by Smith *et al.* (2008) and confirmed in this study (Chapter 3), species extinctions occur even at low application levels.

A second limitation is that I did not calculate livestock production using real animal measures as the plots were too small to support cattle, rather I based my calculations on values from the literature. However, this limitation can also be seen as an advantage in that comparisons can be made between different types of livestock farming. For example, to sustain beef production in the Colt Park trail, organic or high doses of mineral fertilisers are needed to produce the hay quality required for animal breeding (i.e. Richards and Genever 2016; Vickers and Stewart 2016). However, if the class of stock is chosen based on values of crude protein and metabolizable energy of the current forage, other options become apparent – for example all the treatments including no fertiliser addition would be suitable for keeping dairy cows (data from Gammont 2012). In reality it is likely that this area is unsuitable for diary production for a number of reasons, but this line of reasoning may warrant further attention. Thus, an interesting line of enquiry would

be to test whether wild life conservation can be achieved through the better adaptation of farming to the local environment, rather than adapting wildlife conservation to the farming system already in place.

A third limitation is that Colt Park is a small-scale experiment where small plots are adjacent, this enabling easy movement of insects among treatments. However, differences among plots could still be identified, moreover effects at a small scale can translate into patterns seen at the farm scale (e.g. Orford et al. 2016). The fourth limitation is that I assumed no loss of hay during management or due to mould, neither did I consider the effect of cow age on the amount of hay needed. In this sense, the number of cows calculated per hectare are optimistic. Future work could readily accommodate these additional sources of variation by building in variation in the values subjected to randomisation testing. And obviously a future research area would be to test livestock productivity in real animals and include production costs.

What is the optimum fertiliser practice for having both insect conservation and livestock production?

Although, this is a relatively simple attempt to combine the needs of insect and livestock resources. It clearly shows that using artificial fertilisers to achieve a small improvement in livestock production, causes a disproportionately negative impact on diversity in upland grasslands. No fertiliser addition was the best conservation practice for insect resources and insect communities and in terms of productivity, the difference in livestock production between control and the other fertilisers is less than one suckler cow per hectare.

In Europe, grasslands are the result of centuries of farming practices that include the addition of farm yard manure (Poschlod and WallisDeVries 2002) and they are of economic and cultural importance too. For example, in 2017, cattle production for meat contributed £ 2989 million to the national economy of the United Kingdom (DEFRA and National Statistics 2018), and around 0.5% of the English population work on agricultural holdings (DEFRA 2018b). For that reason, the implementation of management practices needs to be established under realistic economic and social criteria as well as for wildlife conservation. That said, my data support the notion of no fertilisation application on hay meadows which have no previous fertiliser history as any type of eutrophication can decrease meadow biodiversity (Andrews and Rebane 1994).

The results of my analysis also encourage the incorporation of areas with no fertilisers under some management schemes, these including the design of agricultural landscapes for biodiversity-based ecosystem services (see Landis 2017), as well as other recommendations to

reduce other environmental problems caused by livestock production (i.e. Muller et al. 2017). While not without its limitations, my approach enables the pros and cons of adding fertilizers on both biodiversity and livestock to be comparatively evaluated and provides a minimum number of useful metrics that can act as responsive indicators of ecosystem service support.

In 2005, Hodgson et al., stated that the maintenance of high biodiversity was incompatible with management for maximum productivity and economic profit. My results suggest that the direct application of artificial fertilisers causes the greatest collateral damage on insect-related ecosystem services and an appropriate evaluation of these disbenefits would reduce the apparent positive effect on the productivity and economic profit. This is especially so, in the current climate when meat consumption is being discouraged by sectors other than the environmental sector, due to its negative consequences on both human health and the environment (Godfray et al. 2018).

In the Colt Park experimental trial, the addition of NPK alone did not offer an improvement over FYM for livestock forage production or in nutritional content when compared to the control. Although, the increase in herbivore abundance following the addition of NPK was as high as the other fertiliser treatments (Chapter 3), the percent parasitism under NPK was the lowest of all the treatments. However, its impacts on insect resources were lower than the rest of the fertiliser treatments. The low increase in livestock production and its impact on percent parasitism make this fertiliser economically and environmentally unviable.

In terms of environmental costs, besides the environmental pollution caused by inorganic fertilisers (Bauer et al. 2016), an impaired feedback between herbivore and parasitoids abundance could lead to an increase in pesticide use. Some neonicotinoids pesticides have been proven to reduce pollination and crop production (Potts et al. 2010, Stanley et al. 2015) and so have been banned by some governments (Butler 2018). This fact highlights the importance of preserving natural pest control. Consequently, the real economic cost of replacing FYM with NPK at the farm level may include the cost of goods such as pesticides with a higher environmental impact.

When NPK is applied in addition to FYM (albeit a rather uncommon practice in farms around Colt Park due to costs) it negatively affects soil micro biota, plant diversity and insect diversity (Smith et al. 2008b, Kidd et al. 2017, Evans and Sanderson 2018). My study shows a similarly negative impact on plants and insects and when NPK+FYM was applied, the negative impacts on insect and insect resources were the greatest. However, when comparing productivity between NPK+FYM and FYM, the “extra” addition of NPK, produced 1.89 kg/h of hay (an average

5% more than FYM), which is enough to feed roughly more one cow during the winter, but with the environmental cost of a 40% decrease in nectar productivity in comparison to FYM.

The massive decline in nectar productivity found in this study and in forb species richness in the previous chapter, are likely to be the cause of the low abundance of bumbles bees observed in this treatment. Pollination is an important ecosystem service worth £1.8 billion a year in the UK (Breeze 2012) and we need a better understanding of the relationship between fertilisers, livestock production and pollinator abundance if we are to maintain livestock production without potentially threatening pollination services. While mass-flowering crops are uncommon in the area around Colt Park, traditional Orchards with fruit trees dependent on insect pollination are more common and are a focus for agri-environment support being part of the traditional farmed landscape.

Farmyard manure was in this sense the best of the fertiliser treatments; however, it needs to be considered that in terms of plant and parasitoid diversity it was not. Nevertheless, in the Colt Park experiment the sowing of legumes has increased plant diversity and fungus:bacteria ratios in soil in this treatment (Smith et al. 2008b); and when comparing the effects of fertilisers when including seed addition, FYM has less impact on plant- flower visitors and plant-herbivore than when artificial fertilisers are added (Chapter 3). Regardless of the legume additions, NPK+FYM still performs poorly compared with the other fertilisers treatments (Smith et al. 2008; see previous chapter) since its effects are strong enough to decrease legumes and remain for longer time (Kidd et al. 2017). Although its moderate impact on insect communities, FYM could have a higher ecological impact than the use of mineral fertilisers. In Colt Park trail the livestock allowed to grass, although it receives low levels of food supplements to avoid eutrophication. It is not organic, so, the FYM contains antibiotic and other substances of veterinary use that can affect other ecosystem services i.e. removal and decomposition of dung (Andrews and Rebane 1994, Hammer et al. 2016).

One big disadvantage of FYM compared to the addition of artificial fertilisers, both in isolation and in combination with FYM, is the lower parasitoid diversity found with FYM. However, parasitoids species represent a single functional group in which diversity is not linked to function or community stability (Rodríguez and Hawkins 2000). Consequently, percent parasitism is a more relevant measure of pest control than diversity, particularly in response to eutrophication where species functional groups respond differently (de Sassi et al. 2012a, Harpole et al. 2016). When considering percent parasitism, the performance of FYM was better than the artificial fertilisers,

whether applied with or without FYM (chapter 3). In this sense, the addition of artificial fertilisers not only reduce resources for pollinators but also risk the efficiency of pest control.

CONCLUSION

Intensive farming practices around the world but particularly in Europe, have ecologically impacted grasslands for decades (Stoate et al. 2009), this promoting extensive research on their environmental impact. This interest has led to a variety of policy regulations on farming practices and has attracted interest from other disciplines such as law, economics and sociology. However, to improve wild life conservation in ago-ecosystems, in the current social, economic and technological context, it is important to integrate the different advances from all these disciplines. Thus, it remains very uncommon to study the effect of fertilisers on both biodiversity and livestock simultaneously, but this is the only way that realistic solutions are going to be found.

Conservation ecologists need to work alongside veterinary professionals and livestock managers in order to devise management approaches acceptable to both parties. In this study, I only evaluated possible effects of two types of fertilizer on two types of insect communities (albeit on a community of 191 species in total). If the direct and indirect consequences on other species are also considered the consequences of fertilisers on the environment could be greater and probably more expensive, especially after accounting for the cost of mitigation actions such as subsidies or restoration programs. Looking forward, we need more studies which link biodiversity and farmers profit margins, which while rare do exist (e.g. Blaauw and Isaacs; Ravetto Enri et al. 2017) and we need more studies which consider multiple ecosystem services. This approach will make the magnitude of the environmental impact more apparent, and potentially, make solutions more straightforward to identify.

Table 4.1. *Individual variables and their classification into groups.*

Variable	Explanation	Group
Diversity of plants	Shannon-wiener index (ln)	**Insect resources**
Forbs species richness	Number of forb plant species (and approximation of diversity of flower resources for pollinators, considering the coevolutionary adaptations between plant-flower visitors).	
Nectar productivity	µl of nectar produced by the total of forbs present in each plot (calculated from Baude *et al.* 2016).	
Abundance of *Bombus* spp.	Number of observations of bumble bees feeding on the plots.	**Insect communities**
Diversity of herbivores	Shannon-Weiner index (ln)	
Diversity of parasitoids	Shannon-Weiner index (ln)	
Diversity of pollinators	Shannon-Weiner index (ln)	
Percent parasitism	Proportion of emergences parasitoids from the total of emergences. It is an indicator of pest control efficiency.	
Ash	No organic matter contents. Values >13 % can cause soil contamination and increase secondary fermentation and risk of listeriosis in livestock.	**Livestock resources**
Crude Protein	Proportion of nitrogen in the hay.	
Neutral Cellulase Gammanase digestibility	Fibre available for digestion in the rumen.	
Oil B	A measure of the percentage of oil in the hay.	
Ruminant Metabolizable energy	Energy available to the cow after accounting for losses in digestion, gases and urine.	
Mean dry weight (2011-2014)	Proportion of the feed that is not water measured in grams of dry hay per m^2.	
Head of suckler cows (600kg)	Number of beef cows that can be fed indoors during the winter (24 weeks) considering an dairy consumption of 7.5kg of hay and assuming no feed losses.	**Livestock production**

Table 4.2. *Results of the Pearson correlation test between variables of hay quality.* Significant correlations are indicated in bold. N=24

	Oil B	**Crude protein (6.25 x N)**	**Ruminant Metabolizable Energy**	**NCGD**	**Ash**	**Mean dry weight g m-2**
Oil B	1	**0.617**	0.3184	0.181	**0.471**	0.11
Crude protein (6.25 x N)	**0.617**	1	**0.477**	**0.402**	**0.728**	0.127
Ruminant Metabolizable Energy	0.3184	**0.477**	1	**0.99**	0.372	0.168
NCGD	0.181	**0.402**	**0.99**	1	0.317	0.156
Ash	**0.471**	**0.728**	0.372	0.317	1	0.173
Mean dry weight g m-2	0.11	0.127	0.168	0.156	0.173	1

Table 4.3. *Rank of the means for each of the individual variables and the general scaled mean when groups of variables are compared*. The rank goes from 4=highest to 1=lowest performance. Colours are according to the rank in a scale that goes from light grey for the lowest performance to dark grey for the highest performance.

	Control	**FYM**	**NPK**	**NPK+FYM**
Diversity of plants	4	2	3	1
Forbs species richness	4	2	3	1
Nectar productivity	4	2	3	1
Abundance of *Bombus* spp.	4	3	2	1
Diversity of herbivores	2	3	1	4
Diversity of parasitoids	3	1	2	4
Diversity of pollinators	4	2	3	1
Percent parasitism	2	4	1	3
Ash	4	2	1	3
Crude Protein	1	3	2	4
Neutral Cellulase Gammanase digestibility	2	4	1	3
Oil B	1	4	3	2
Ruminant Metabolizable energy	2	4	1	3
Mean dry weight (2011-2014)	1	3	2	4
Heads of Suckler cows (600kg)	1	3	2	4
Mean	**3**	**4**	**2**	**1**

Table 4.4 *Probability that the observed mean rank is higher than the mean rank calculated with the randomization test for each variable and agroecosystem service*. N=24

Variable	Control	FYM	NPK	NPK+FYM
Variables analysed individually				
Plant diversity	0.8307	0.2206	0.5727	0
Forbs species richness	0.9252	0.236	0.6576	0
Nectar productivity	0.9174	0.2204	0.5305	0
Abundance of *Bombus* spp.	0.9544	0.4979	0.2584	0
Diversity of Herbivores	0.2929	0.5384	0	0.8604
Diversity of Parasitoids	0.4596	0	0.2234	0.9129
Diversity of Pollinators	0.8257	0.2274	0.587	0
Parasitism rate	0.2113	0.8816	0	0.599
Ruminant ME	0.2416	0.9122	0	0.5712
Crude Protein	0	0.6829	0.2035	0.9005
Moisture	0	0.5419	0.9024	0.126
Ash	0.7521	0.2397	0.211	0.5199
Oil B	0	0.9093	0.5841	0.2232
NCGD	0.2667	0.8995	0	0.5679
Carbon/Nitrogen 2011-2015	0.2016	0.8047	0.5306	0
Mean dry weight 2011-2015	0	0.5953	0.2592	0.952
Heads of Suckler cows (600kg)	0	0.5733	0.3019	0.9504
Variables analysed by group				
Insect resources	0.9558	0.1779	0.635	0
Insect communities	0.8802	0.68	0	0.1402
Livestock resources	0	0.9354	0.1125	0.7819
Mean of the three groups	0.5737	0.9285	0.1154	0

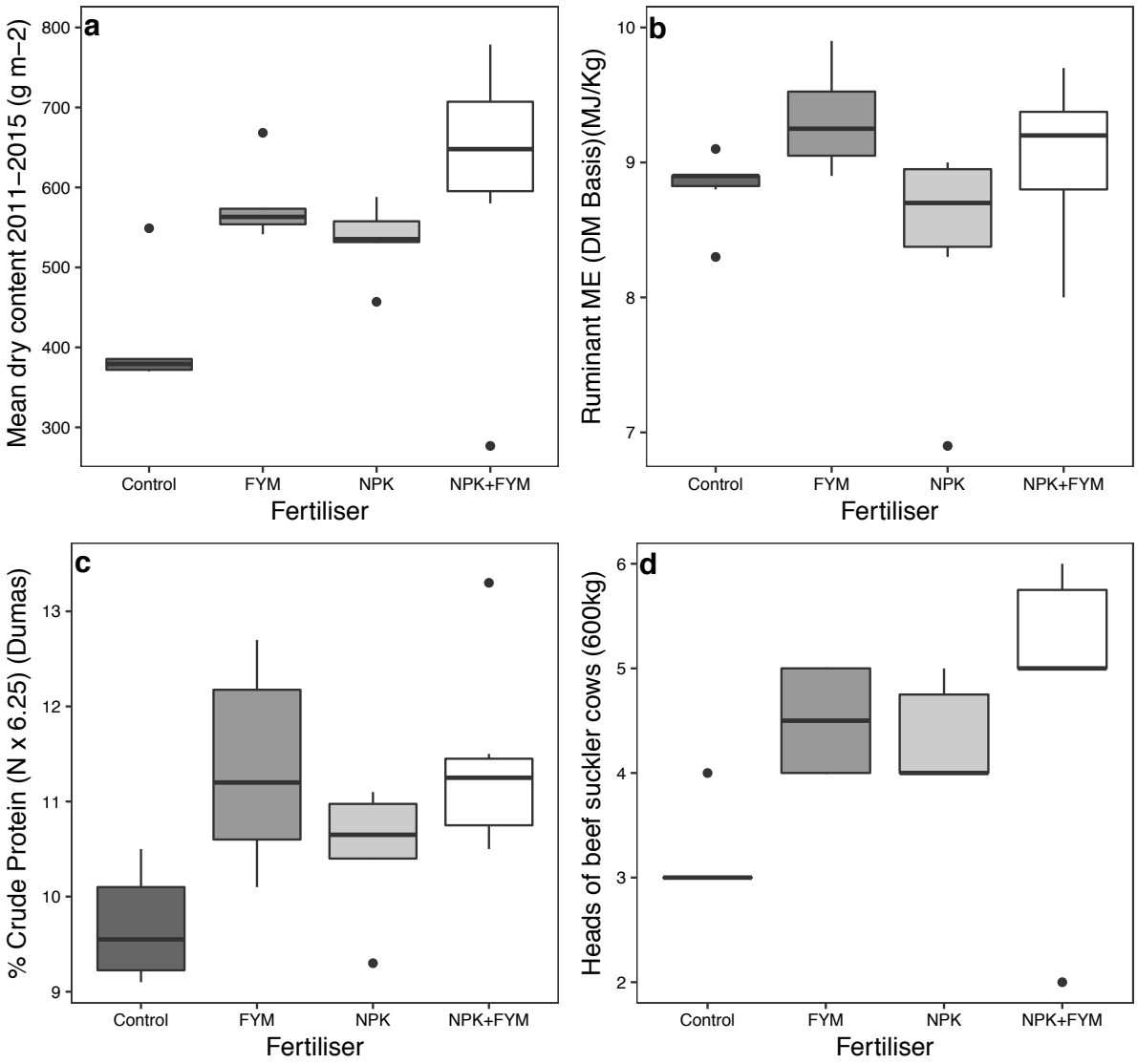


Figure 4.1. *Data distribution for each of the fertiliser treatments* for: a) Mean dry content 2011-2015, b) Ruminant Metabolizable energy, c) percent of crude protein and d) head of beef suckler cows. N=24

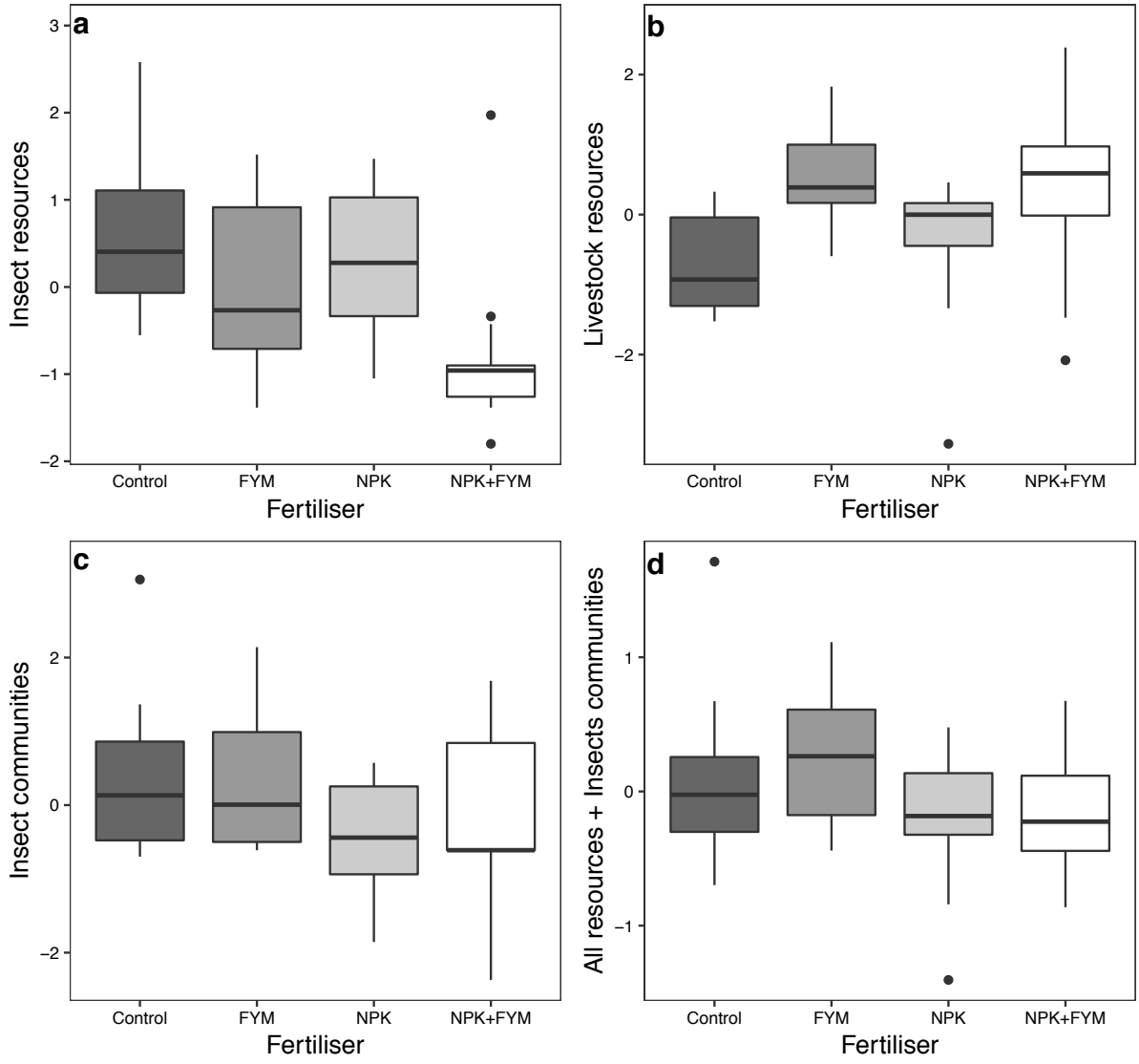


Figure 4.2. *Data distribution of each category for each of the fertiliser treatments:* a) insect resources, b) Livestock resources, c) Insect communities and d) all categories considered together: all resources (insect +livestock) and insect communities. N=24

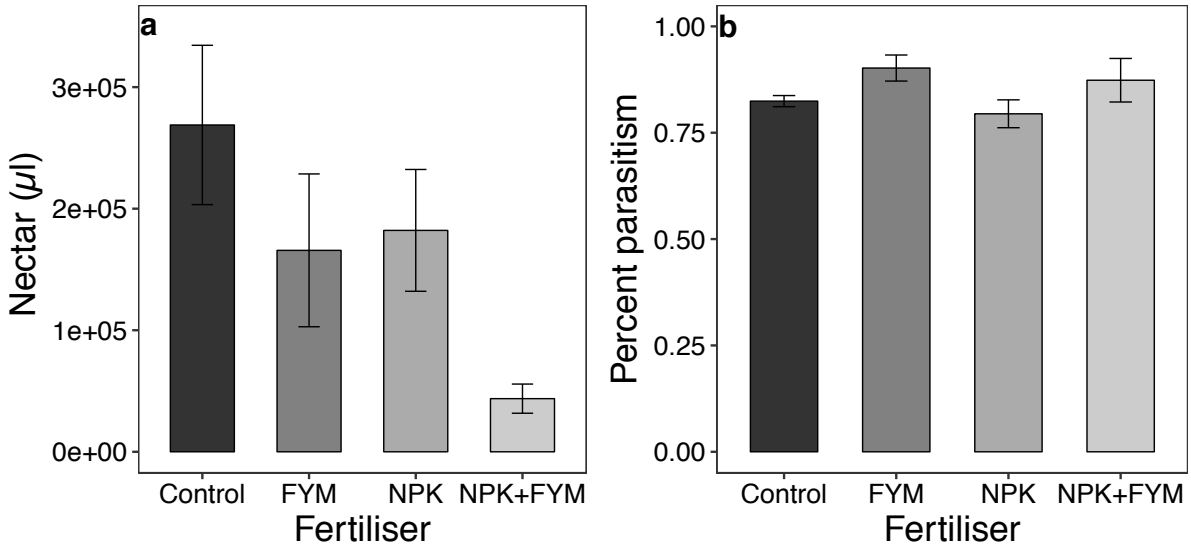


Figure. 4.3. *Mean values among fertiliser treatments in:* a) nectar productivity, b) percent parasitism. N=24.

SUPPLEMENTARY INFORMATION

Calculation of the hay quality variables

Methods were provided by Sciantec Analytical services:

Ruminant metabolizable energy (ME_{dm}): calculated using the formula:

$$ME_{dm} = (0.14 \times NCGD_{dm}) + (0.25 \times OAH_{dm})$$

Where $NGCD_{dm}$ is neutral cellulase gammanase digestibility of the dry matter of the sample and OAH dm is the amount of oil B of the dry matter of the sample.

Crude Protein: calculated by multiplying the amount found of nitrogen by 6.25 (average content of nitrogen in proteins) under the method of Dumas (see Wolfgang and Gunnar 1983).

Moisture: loss in weight of the sample after heating the sample at 103-105°C for 2 hours 45 minutes ± 15 minutes

Ash: residue of the samples after it has been incinerated at 510°C for at least 4 hours.

Total oil (Oil B): after the oil is extracted with ether the sample is boiled in hydrocholoric acid to release the bound fat which is retained by a filter paper and then measured.

Neutral cellulase gammanase digestibility: amount of fibre of the sample is extracted by using the enzymes cellulase and gammanase. The amount of sample not digested by the enzymes is then measured.

Supplementary information table. S.4.1. *Results of the hay quality analysis.* All results are in percentage. Ash content is the difference between the original value and limit value where ash content has a negative effect on soil and animal digestion (13%).

Sample	Ruminant metabolizable energy	Crude Protein	Moisture	Ash	Total Oil content	Neutral cellulase gammanase digestibility	C:N_(2011-14)
Control							
1	8.9	9.2	7.5	7.4	2.58	58.8	29.16
2	8.9	9.3	6.1	6.6	2.82	57.9	25.51
3	8.3	10.5	7.2	6.6	3.29	52.9	29.78
4	8.9	9.1	6.2	6.9	3.05	58	30.35
5	9.1	10.2	7	6.3	3.28	58.9	27.99
6	8.8	9.8	6.3	7	2.81	57.7	27.5
Farm yard manure							
1	9.3	10.4	7.4	6	3.43	59.7	28.18
2	9	11.2	6.7	7.6	3.4	58.1	28.55
3	8.9	10.1	7.1	6.1	3.01	57.6	27.62
4	9.6	12.5	7	7	3.7	61.3	29.13
5	9.2	11.2	7	7.3	3.6	58.9	28.73
6	9.9	12.7	7.2	5.7	3.7	63.8	32.11
N:P:K & farm yard manure (NPK+FYM)							
1	9.3	10.6	7.2	6.5	3.17	60	27.27
2	9.1	10.5	7.4	7.2	3.45	58.1	28.92
3	8.7	11.5	5.4	6.3	2.64	57.3	28.8
4	9.7	11.3	5.9	7.6	3.37	62.8	28.22
5	9.4	13.3	7.1	4.9	3.48	60.3	26.83
6	8	11.2	7.4	7.8	3.46	50.6	29.67
N:P:K							
1	8.8	11.1	6.2	6	3.42	56.3	29.98
2	6.9	9.3	7.8	7	3.15	43.2	32.8
3	9	10.4	7.3	7.6	3.46	57.8	27.57
4	9	10.9	7.6	5.5	3.63	57.4	27.91
5	8.3	11	7.5	8	3.19	52.9	26.64
6	8.6	10.4	8	5.6	3.28	55	28.57

CHAPTER FIVE

DISCUSSION

INTRODUCTION

Given its well-documented negative effects on biodiversity, eutrophication has been the focus of multiple studies. Most research however has been about evaluating a single nutrient or a single focal species. In contrast to most previous approaches, my thesis has explored the mechanisms through which eutrophication affects whole communities of interacting species. In brief, I found that the non-random effects of eutrophication on the plant community composition affect several aspects of the insect communities from changes in their abundance to the structure of their ecological networks. Where responses of the insect communities were guild-specific and variable depending of the source of enrichment. I also observed that such changes are expected to increase in the future, so the implementation of sustainable practices are needed particularly in agro-ecosystems where negative effects exceeds the benefits of eutrophication.

In Chapter 2, I showed that although nitrogen enrichment is an important global driver of loss in diversity, under natural soil conditions multiple nutrients impact insect communities and their consequences vary within insect groups. I found that soil nutrients have additive effects on network structure and that soil fertility in general, rather than nitrogen deposition alone, leads to an increase in generalist species. I also predicted that generalism will increase further under future nutrient enrichment. Given this tendency to generalisation in my networks, I can also therefore predict that homogenization of the communities is expected under eutrophication, and this is regardless of the source of nutrient enrichment. This effect being in keeping with Laliberté & Tylianakis 2010 who stated that homogenisation is an expected consequence of human disturbance on ecological networks. Although, the data and statistical power of my analysis in Chapter 2 is limited, the approach will help to understand the effects of nitrogen deposition on insect networks and how local soil fertility affects the magnitude of such effects. Hopefully my findings will help to better design nutrient deposition experiments and inform the modelling of future scenarios of nutrient enrichment.

Chapter 3 - the Colt Park Trial data – here I show that eutrophication affects the structure of the different interaction networks. Moreover, I explain how changes in plant community are transmitted upwards through the network. This chapter will help ecologists and land managers to understand the process of nutrient enrichment and hopefully inspire further research at the community level. In the introduction, I discussed how eutrophication could affect the complexity of the networks by decreasing species richness. In Chapter 3, I found that the changes in food web structure were not due to a loss of species, but to changes in plant community triggered by eutrophication which affected the abundance of species and thereby their likelihood of interacting

with other species. A common response of grasslands to nutrient enrichment is the loss of forbs and this loss of floral resources has been widely discussed as a cause of the pollinator crisis (Potts et al. 2010, Baude et al. 2016). My data confirmed that the loss of floral and nectar resources indeed affects the structure of plant-flower visitor networks. I also observed that similarly, the loss of forbs affects the structure of plant-herbivore-parasitoid networks although their lower dependence on forbs resources led to a difference response. This result shows the importance of studying different groups simultaneously.

Finally, chapter 4 asks whether the environmental consequences of increasing of livestock production are compatible with a sustainable agriculture and the answer is very likely they are not. My results showed that a 40% loss nectar is the consequence of a 5% increase in productivity. A large concern of reducing fertiliser use though, is a decrease in food production. Even at current levels these can be insufficient for human needs, particularly in developing countries and emerging economies that also happen to be the most overpopulated area in the planet (FAO 2015a). Even in the UK, Qi et al. 2018 state that fertiliser regulation means that agroecosystems are below their maximum productivity. My results showed that hay productivity and nutrient content do not increase at the same rate with the addition of fertilisers. Thus, control plots were not so different in terms of productivity than the fertilised ones, supporting the idea that biodiversity leads to high productivity in grasslands (Tilman 1999). However, the nutritional content of the hay from control plots was insufficient to support the nutritional needs of livestock. In this sense achieving higher nutritional content should perhaps be considered at least as important as achieving higher productivity. This is a field that seems quite promising particular given the recent advances in improving the efficiency of plant in the intake of nitrogen (See Varala et al. 2018) and nutrient quality (Barrett et al. 2015), modifications that could allow a reduction in fertiliser application rates. The next step for ecologists in this context, is to test whether such advances benefit wild life conservation in agroecosystems.

When considering each insect communities separately, I found that flower visitors were the most affected given the decreased in floral and nectar resources. Within the flower visitors, bumble bees highly decline in abundance with nutrient enrichment as reported by Stevens et al. 2018. However, this is the first time that effects are recorded from an experimental study. Additionally, I found a tendency in generality in plant-flower visitor network. Burkle et al. 2008 also found such tendency however this is the first study that shows that the successional process due to eutrophication promotes asymmetry in plant-flower visitor networks. This asymmetry could mean an increase in competition in flower visitors community that could provoke future

extinctions events. More important, I observed that a high decline in nectar productivity and loss of bumble bees are done to achieve a modest increase in livestock productivity.

For herbivores, I observed that increase of generalist species with eutrophication not only occur in communities of Lepidoptera species as reported by Pöyry et al. 2017, but also in different insect order (i.e. Diptera, Coleoptera) and different feeding habits (external and internal feeding). I observed that only the abundance of herbivores feeding on species beneficiated by eutrophication increased, so the idea that nutrient enrichment increases abundance of herbivores is a generalization that hardly represent the responses of herbivores at community level. Previous works have reported mainly positive effects of eutrophication such as increase in abundance or increase in palatability (Stiling & moon 2005; Throop & Lerdau 2004), but this is the first study that shows that the decline in forbs species also increases the generality of herbivore species although they can feed on grasses and other competitive species.

For parasitoids, I found that despite the weak effect or no effect of eutrophication on reported in previous studies (de Sassi et al. 2012a; Scherber et al. 2010). Parasitoids are also affected by eutrophication showing an increase in generality and increased in abundance. To my knowledge, this is the first study that reports that the increase in generality is given by two mechanisms: a) by attacking new species of herbivores and b) by increasing the number of attacks to existing species. Finally, I found that the addition of inorganic fertilisers diminishes the percent of parasitism in comparison to organic fertilisers which means a lower pest control efficiency when adding inorganic fertilisers.

WHAT COULD HAVE BEEN DONE BETTER?

In chapter two, while I used a novel criterion for the removal of species, my approach could be further improved by using a more sophisticated method of implementing species loss. I used the classical topological removal of species which did not consider potential changes in feeding preferences and I also assumed that extinction occurs only when all resources species are lost (whereas in reality it probably happens when resource species become rare). Differences in species efficiency need to be included too (Vieira and Almeida-Neto 2015) as ecosystem services can be affected even if some species remain, for example Traveset et al. 2017 observed that the seed set of plant species diminished even when some of their pollinators remained following a disturbance event.

Currently there is a considerable interest in the use of Bayesian statistics. In the field of ecological networks, the use of Bayesian network approach could provide a versatile method for

testing the robustness of ecological networks. Bayesian networks allows the study of different trophic levels and guilds simultaneously under more realistic scenarios than traditional robustness measures that are calculated exclusively from species interactions (Eklöf et al. 2013). It also allows the rewiring of network layers (i.e. species that in immature states are herbivores, predators or parasitoids but pollinators as adults). They are also computationally simpler enabling large networks to be subjected to complex extinction scenarios. A future direction would be to calculate the robustness of multiple networks (i.e. both trophic and mutualistic networks) using species traits and environmental factors to build probabilities of extinction using a Bayesian framework. If this method was applied in different habitats, each of which provided data on their biotic interactions, it would be possible to create a map showing where ecosystem services are being affected by eutrophication. This map would be similar to the "Ecosystem service interactions-spatial interactive tool for visualising ecosystem service interactions" developed by the Biodiversity & ecosystem service sustainability (BESS) et al. 2014 which allows to visualise how different management intervention could change interaction in ecosystem services.

In the Colt Park chapter, I consider changes in host availability, however changes in host quality could also affect primary and secondary consumers. Lavorel et al. 2013 offer a good theoretical model of possible responses on multiple trophic levels to changes to plant quality. However, it has not yet been tested using an experimental approach. It would be interesting to test if eutrophication also affects the rewiring of interactions by changing host quality. Such rewiring, together with low host availability, could result in species extinctions. Originally, I planned to include this variation in host quality and its effects on insect communities. Thus, I considered measuring nectar content for the plant-flower visitor networks and leaf dry matter content for the plant-herbivore-parasitoid network. However, I could not find a plant species with a high enough nectar production to test for differences under the different treatments or a representative number of plant species that grow in all treatments to test variation in dry matter content at community level.

I used barcoding for parasitoid identification which is still rather unusual in community wide studies. This approach comes with some large advantages, for example there is a real bottleneck with parasitoids taxonomists worldwide and this method was used as the taxonomist used in previous projects could not identify my specimens before the end of my funding! Barcoding is fast in comparison. However, this method still requires a successful insect rearing and rearing success varies with insect species. For example, Lepidoptera survival is usually around 50% (e.g. Macfayden et al. 2009), a figure in keeping with my own rearing success, and larval death represents a loss of information in terms of both herbivore and parasitoid species

identification. Barcoding of the herbivores together with metabarcoding are probably a good solution for this, although still expensive and still under improvement. Metabarcoding allows the identification of host plants and parasitoids by using plant DNA from the gut content of the herbivores and parasitoid DNA from where ever they are feeding inside the caterpillar. However, these approaches come with complications of their own, for example identifying two species of parasitoids inside a larva, of which only one would actually emerge if reared. In this sense, molecular information does not replace traditional methods, but complements them (Wirta et al. 2014).

Finally, for the question of the optimum fertiliser application for insect conservation, a question addressed in Chapter four, it would be interesting to undertake a research project at a much larger scale and at multiple sites, perhaps even in different countries. In this experiment livestock productivity and ecosystem services in grasslands would be measured considering, for example, the balance between the livestock production profit margins and the cost of different mitigation practices needed to reduce the environmental consequences of eutrophication. In this thesis I combined stock production, eutrophication and biodiversity for the first time, it would be highly informative to incorporate economics too. Another, interesting approach would be to include different scenarios of meat consumption, for example comparing current consumption to different levels of vegetarianism or flexitarianism (reducing meat consumption). But where, collateral effects such as the need of producing more vegetable protein in place of meat consumption are also considered.

WHAT IS NEXT?

Nutrient enrichment is a research field that offers many future research projects. Currently, there is much data on its impact on plant communities, soil microbiota and single species systems, but rather little data on its impact on animal communities. Integrative, applied and multitrophic studies are needed in both temperate and tropical areas. Looking at differences in nutrient enrichment in different areas of the world would be an exciting approach. For example, considering the fact that nutrient enrichment is a global phenomenon where the consequences of nutrient changes go far beyond the place where the nutrients are released or regulated, thereby causing environmental impacts in other regions of the planet (i.e. Thomas et. al. 2016) . When assessing the effects and future tendency of eutrophication we need to consider the global landscape mosaic of nutrient enrichment in which there are places highly affected by nutrient enrichment and places where fertilisers are needed (FAO 2015b) as well as the additive effects

atmospheric nutrient enrichment. This information is needed in order to be able to establish policies with the lowest negative side effects.

In this thesis, I focused on the bottom-up effects of eutrophication. The reason for this decision was the considerable quantity of research on the impact of eutrophication on plant communities which provided the basis for many of my hypotheses predicting the response of insect community. However, top-down effects also play an important role during the process of eutrophication. For example, Siemann 1998 found that the compensatory effects of parasitoids on herbivore populations maintained plant productivity, even when the number of plant species declined due to fertiliser addition. The assessment of top-down effects requires a different experimental approach to the one I used though, for example the inclusion of plots where natural enemies are excluded.

A further area of future research concerns the recovery of the interaction networks. In a long term experiment Clark & Tilman 2010 observed that plant diversity recovered when nutrient application ceased. A similar effect was found by Lepš (2014) who reported that the removal of dominant plants under eutrophication helped to reduce species loss following 15 years of fertiliser addition. It would be very interesting to test whether vegetation recovery also restores the insect communities and their ecosystems services.

CONCLUSIONS

Eutrophication is a process that has impoverished different habitats around the world. In this thesis, I provided evidence of its effects on different types of interaction networks. I also highlighted the utility of interdisciplinary approaches to attack environmental problems, bringing together the fields of nutrient enrichment and ecological networks. Community ecology is an integrative discipline where by using an interdisciplinary approach, we may be able to mitigate current environmental issues such as eutrophication. To succeed though, we need to consider the aims and aspirations of both conservation biologists and farmers and ideally find solutions acceptable to both.

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APPENDICES

APPENDIX 1 List of species in each of the plant-insect-inset networks from Bristol

The letter corresponds to the name of the place and the number indicates one of the two plots within the same field site. A: Ashton, B: Brandon Hill, C: Crabtree, D: The Downs, O: Oldbury Court and T: Troopers Hill.

Table 1 List of plant species

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
Agrostis capillaris L.										x		
Agrostis stolonifera L.				x								
Anthoxanthum odoratum L.				x								
Arrhenatherum elatius (L.) P.Beauv. ex. J. Presl & C. Presl.	x	x	x	x							x	
Asteraceae sp.							x		x	x		
Bellis perennis L.								x				
Bromus erecta Huds.						x		x			x	
Cardamine pratensis L.						x						
Centaurea nigra L.			x		x		x	x				
Circaea lutetiana L.				x								
Conopodium majus (Gouan) Loret.		x										
Crataegus monogyna Jacq.	x											
Crepis capillaris (L.) Wallr.				x				x				
Crepis versicaria/biennis									x			
Dactylis glomerata L.		x		x							x	
Dactylorhiza fuchsii (Druce) Soó	x											
Erica tetralix L.												x
Geranium molle L.											x	
Geranium pratense L.			x									
Grass	x	x	x	x	x	x	x	x	x	x	x	
Helianthemum nummularium L.Mill							x		x			
Heracleum sphondylium L.	x	x	x	x							x	x

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
Hieracium pilosella L.							x				x	x
Holcus lanatus L.		x		x		x		x	x	x	x	
Hypericum maculatum Crantz					x	x						
Hypochaeris radicata L.	x		x	x	x	x	x	x	x	x	x	x
Knautia arvensis (L.) Coult.					x	x						
Lathryrus pratensis L.	x				x							
Leontodon hispidus L.	x	x			x	x	x					
Leucanthemum vulgare Lam.			x	x								
Lolium perenne L.										x		
Lotus corniculatus L.	x		x		x	x	x	x			x	
Plant 11							x					
Plant 12							x					
Plantago lanceolata L.	x	x	x		x	x	x	x	x	x	x	
Potentilla reptans L.		x				x						
Primula veris L.			x									
Ranunculus bulbosus L.	x	x	x		x	x	x	x	x	x	x	x
Ranunculus repens L.	x	x	x	x	x	x		x			x	x
Rhinanthus minor C.C. Gmel	x		x				x	x				
Rubus fruticosus L.	x			x								
Rumex acetosa L.	x		x			x						
Solidago virgaurea L.												x
Stachys officinalis (L.) Trevis			x									
Taraxacum officinale aggr.	x	x	x	x			x	x	x	x		
Thymus praecox subs. polytrichus (A. Kern. ex Borbás) Jalas					x							
Trifolium hybridum L.									x			
Trifolium pratense L.	x	x	x		x	x	x	x			x	
Trifolium repens L.	x	x			x		x	x	x	x	x	
Trifolium sp.			x							x	x	

Table 2 Species of flower visitors

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
COLEOPTERA												
Anaspis sp.	x											
Blepharidopterus angulatus (Fallén, 1807)							x					
Byturus ochraceus (Scriba, 1790)			x	x								
Cantharis rustica Fallén, 1807		x										
Crepidodera transversa (Marsham, 1802)	x					x	x	x				
Cryptocephalus aureolus Suffrian, 1847					x	x						
Gymnetron pascuorum (Gyllenhal, 1813)	x											
Malachius bipustulatus (Linnaeus, 1758)			x			x			x			
Meligethes aff. *aeneus* (Fabricius, 1775)	x			x			x	x	x			
Oedemera lurida (Marsham, 1802)	x			x	x	x					x	
Oedemera nobilis (Scopoli, 1763)	x		x	x	x	x	x	x			x	
Olibrus liquidus Erichson, 1845				x							x	x
Phyllobius virideaeris (Laicharting, 1781)		x										
Phyllopertha horticola (Linnaeus, 1758)	x											
Prosternon tesselatum (Linnaeus, 1758)											x	
Rhagonycha fulva (Scopoli, 1763)											x	
DIPTERA												
Bicellaria sulcata (Zetterstedt 1842)								x				
Botanophila sp.	x	x										
Botanophila striolata (Fallén, 1824)				x								
Botanophila varicolor (Meigen, 1826	x											
Cheilosia albitarsis (Meigen, 1822)	x	x	x	x	x		x				x	
Cheilosia albitarsis/ranunculi		x										
Cheilosia antiqua (Meigen, 1822)		x										

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
Cheilosia proxima (Zetterstedt, 1843)						x						
Chloromyia formosa (Scopoli, 1763)		x		x								
Delia sp.				x								
Empis albinervis (Meigen, 1822)	x						x	x				
Empis caudatula Loew, 1867		x						x				
Episyrphus balteatus (De Geer, 1776)											x	
Euleia heraclei (Linnaeus, 1758)											x	
Eumerus strigatus Meigen, 1822											x	
Fannia sp.											x	
Helophilus hybridus Loew, 1846			x									
Heterostylodes nominabilis (Collin, 1947)												x
Lucilia sericata (Meigen, 1826)											x	
Melanostoma mellinum (Linnaeus, 1758)						x						
Merodon equestris (Fabricius, 1794)			x								x	
Pegoplata aestiva (Meigen, 1826)	x					x					x	x
Phaonia incana (Wiedemann, 1817)	x											
Pollenia amentaria (Scopoli, 1763)	x											
Lucilia sericata (Meigen, 1826)											x	
Pollenia pediculata Macquart, 1834												x
Scathophaga stercoraria (Linnaeus, 1758)		x										
Sicus ferrugineus (Linnaeus, 1761)								x				
Siphona geniculata (De Geer, 1776)		x			x							
Sphaerophoria sp.											x	
Syritta pipiens (Linnaeus, 1758)							x					
Syrphus ribesii (Linnaeus, 1758)	x			x			x			x		
Tachinidae sp.											x	
Thecophora atra (Fabricius, 1775)					x							
Xylota abiens Meigen, 1822						x						
Zophomyia temula (Scopoli, 1763)		x										
Miridae sp.							x					

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
HYMENOPTERA												
Andrena cineiaria (Linnaeus, 1758)		x										
Andrena labiata Fabricius, 1781											x	
Andrena minutula (Kirby, 1802)							x					
Andrena nitida (Müller, 1776)				x						x		
Andrena semilaevis Pérez, 1903	x											
Apis mellifera Linnaeus, 1758	x		x	x	x			x	x	x		
Bombus campestris (Panzer,1801)										x		
Bombus hortorum (Linnaeus, 1761)			x				x	x			x	x
Bombus lapidarius (Linnaeus, 1758)		x	x	x	x	x	x	x	x	x	x	x
Bombus lucorum (Linnaeus, 1761)		x			x	x	x		x			
Bombus pascuorum (Scopoli, 1763)	x		x		x	x	x	x	x	x	x	
Bombus pratorum (Linnaeus, 1761)	x							x				
Bombus terrestris (Linnaeus, 1758)		x		x	x	x	x	x	x	x	x	
Braconidae sp.1						x						
Braconidae sp.2											x	
Cephus pygmaeus (Linnaeus, 1767)						x					x	
Colletes daviesanus Smith, 1846			x									
Halictus tumulorum (Linnaeus, 1758)			x	x			x	x	x	x	x	
Ichneumonidae sp. 3		x										x
Ichneumonidae sp. 4			x									
Lasioglossum calceatum (Scopoli, 1763)	x	x	x	x		x		x		x		
Lasioglossum fulvicorne (Kirby, 1802)								x				
Lasioglossum leucozonius (Schrank, 1781)		x										
Lasioglossum morio (Fabricius, 1793)			x	x						x		
Lasioglossum smaethmanellum (Kirby, 1802)			x	x			x					
Lasioglossum villosulum (Kirby, 1802)				x					x			x
Lindenius albilabris (Fabricius, 1793)		x										
Macrophaga sp.			x									

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
Macrophaga sp.			x									
Megachile willoughbiella (Meidell, 1944)											x	
Nomada fabriciana (Linnaeus, 1767)				x								
Osmia bicolor (Schrank, 1781)		x										
Osmia leaiana (Kirby, 1802)			x						x			
Panurgus banksianus (Kirby, 1802)											x	x
Sphecodes monilicornis (Kirby, 1802)					x							
Tenthredo arcuata Foster, 1771	x		x	x	x	x	x	x			x	
LEPIDOPTERA												
Chrysoteuchia culmella (Linnaeus, 1758)		x										
Cosmopterix schmidiella Frey, 1856	x			x								
Euclidia glyphica (Linnaeus, 1758)	x					x	x	x			x	
Maniola jurtina (Linnaeus, 1758)	x				x	x			x			
Melanargia galathea (Linnaeus, 1758)	x				x	x						
Polyommatus icarus (Rottemburg, 1775)			x		x	x						
Thymelicus sylvestris (Poda, 1761)					x							
Zygaena filipendulae (Linnaeus, 1758)	x	x	x		x	x					x	

Table. 3 Species of herbivores

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
External feeders												
COLEOPTERA												
Curculionidae sp.											x	
Hypera punctata Fabricius, 1775			x							x		
Hypera sp.											x	
Subcoccinella vigintiquattuorpunctata (Linnaeus, 1758)	x		x		x			x	x		x	
LEPIDOPTERA												
Agonopterix arenella ([Denis & Schiffermüller], 1775)					x		x					
Agrotis exclamatios (Linnaeus, 1758)									x			
Apamea unanimis (Hübner, [1813])										x	x	
Callimorpha dominula (Linnaeus, 1758)	x											
Callistege mi (Clerck, 1759)						x	x					
Cnephasia sp.	x	x			x	x	x	x	x			
Cnephasia sp.2							x		x			
Cnephasia stephensiana (Doubleday, [1849])		x			x					x		x
Diachrysia chrysitis (Linnaes, 1758)			x									
Diacrisia sannio (Linnaeus, 1758)					x							
Hecatera bicolorata (Hufnagel, 1766)				x				x				
Lathronympha strigana (Fabricius, 1775)					x	x						
Lepidoptera sp.											x	
Maniola jurtina (Linnaeus, 1758)	x			x	x	x		x	x		x	
Melanargia galathea (Linnaeus, 1758)					x	x			x			
Mesapamea secalis (Linnaeus, 1758)			x	x	x					x		
Mompha langiella (Hübner, 1796)				x								
Mythimna impura (Hübner, [1808])		x										
Noctuidae sp.									x		x	

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
Orthosia incerta (Hufnagel, 1766)	x						x					
Thymelicus sylvestris (Poda, 1761)	x											
Tortricidae sp.	x	x			x		x	x	x		x	
Tortricidae sp.2							x					
Zygaena filipendula (Linnaeus, 1758)	x		x		x	x					x	
Internal feeders (Leaf miners)												
DIPTERA												
Agromyza albipennis Meigen, 1830				x						x		
Agromyza frontella (Rondani, 1875)	x										x	
Agromyza idaeiana Hardy, 1853						x						
Agromyza nana Meigen, 1830		x	x		x		x	x	x	x	x	
Aulagromyza similis (Brischke, 1880)					x	x						
Chromatomyia milii (Kaltenbach, 1864)				x								
Chromatomyia nigra (Meigen, 1830)	x	x	x	x		x		x	x	x	x	
Chromatomyia paracillata Godfray, 1985			x									
Chromatomyia sp.				x								
Chromatomyia sp.1	x											
Chromatomyia sp.2			x									
Chromatomyia syngenesiae Hardy, 1849a	x		x					x	x	x		
Lyriomyza centaureae (Meigen, 1830)					x							
Lyriomyza flaveola (Fallén, 1823a)										x		
Megaselia sp.	x											
Ophiomyia labiatarum Hering, 1937			x									
Pegomya bicolor (Wiedemann, 1817)	x		x			x						
Phytomyza aff. *fallaciosa* Brischke, 1880		x										
Phytomyza notata Meigen, 1830		x										
Phytomyza plantaginis Goureau, 1851	x	x	x		x	x	x	x	x	x	x	
Phytomyza ranunculi (Schrank, 1803)		x	x	x	x	x	x	x			x	

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
Phytomyza ranunculivora Hering, 1932		x	x		x	x					x	
Phytomyza sp.							x		x			
Phytomyza sp.2			x		x			x				
Phytomyza sphondylii Robineau-Desvoidy, 1851	x										x	
Phytomyza sphondyliivora Spencer, 1957a	x		x	x							x	
HYMENOPTERA												
Fenella nigrita Westwood, 1839		x										
LEPIDOPTERA												
Aspilapteryx tringipennella (Zeller, 1939)	x	x	x		x	x			x		x	
Ectoedemia septembrella (Stainton, 1849)						x						
Elachista sp.			x									
Stigmella aurella (Fabricius, 1775)				x								

Table 4 Species of parasitoids

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
BRACONIDAE												
Apodesmia sp.BOLDADH6245			x								x	
Apodesmia sp.BOLDADH7933	x											
Braconidae sp.BOLD:ADH7741				x								
Braconidae.sp.BOLD:ADH9685								x				
Chorebus aphantus				x								
Chorebus dagda (Nixon, 1943)	x											
Chorebus fallaciosae Griffiths, 1967				x							x	
Colastes braconius Haliday, 1833				x								
Cotesia sp.					x							
Cotesia telengai (Tobias, 1972)				x								
Cotesia zygaenarum (Marshall, 1885)											x	
Dacnusa macrospila (Haliday, 1839) sp.2		x				x						
Dacnusa maculipes Thomson, 1895	x	x	x	x		x		x		x	x	
Dacnusa plantaginis Griffiths, 1967	x	x	x		x	x		x			x	
Dacnusa sp.BOLD:ACK3602			x									
Dacnusa tarsalis Thomson, 1895			x	x								
Microgaster sp.			x								x	
Microplitis sp.				x								
Opius sp.BOLD:ADH6245											x	
Phaedrotoma sp. 2 near exigua			x			x		x			x	
Phaedrotoma sp.1 near exigua	x	x	x		x	x	x	x			x	
Xenarcha sp.		x										
CHALCIODEAE												
Chalciodeae sp.1	x											
Chalciodeae sp.2			x									
Chalciodeae sp.3				x								
Chrysocharis pubicornis (Zetterstedt, 1838)			x	x	x						x	
EULOPHIDAE												
Chrysocharis sp.		x										
Chrysocharis sp.1											x	
Chrysocharis sp.2			x	x								
Chrysocharis viridis (Nees, 1834)			x	x				x				

Species	A1	A2	B1	B2	C1	C2	D1	D2	O1	O2	T1	T2
Diglyphus isaea (Walker)	x	x	x	x				x			x	
Eulophidae sp.1					x							
Hemiptarsenus fulvicollis Westwood, 1833					x							
Hemiptarsenus sp.1		x								x	x	
Hemiptarsenus sp.2			x							x	x	
Hemiptarsenus sp.BOLD:ACL3504	x		x			x		x			x	
Hemiptarsenus unguicellus Westwood, 1833				x						x		
Pediobius metallicus (Nees, 1834)			x	x				x			x	
Pnigalio.sp.2	x										x	
ICHNEUMONIDAE												
Diadegma fenestrale (Holmgren, 1860)	x				x							
Diadegma sp.						x						
Erigorgus cerinops (Gravenhorst, 1829)				x								
Ichnemunidae sp.2				x								
Ichnemunidae sp.3					x							
Ichneumonidae sp.				x								
Itoplectis maculator (Fabricius, 1775)					x							
Mesochorus sp.											x	
PTEROMALIDAE												
Miscogaster maculata Walker, 1883			x	x			x			x		
Pteromalidae.sp.3				x		x						
Seladerma sp.BOLDAC17664			x									
TETRACAMPIDAE												
Epiclerus panyas Walker, 1839					x						x	

Appendix 2 -List of species collected in Colt park experiment

Table 1. List of plant species

Order	Family	Specie
Apiales	APIACEAE	*Anthriscus sylvestris* (L.) Hoffm.
Apiales	APIACEAE	*Conopodium majus* (Gouan) Loret
Asparagales	ORCHIDOIDEAE	*Dactylorhiza fucsii* (Druce) Soó
Asterales	ASTERACEAE	*Bellis perennis* L.
Asterales	ASTERACEAE	*Centaurea nigra* L.
Asterales	ASTERACEAE	*Hypochaeris radicata* L.
Asterales	ASTERACEAE	*Leontodon hispidus* L.
Asterales	ASTERACEAE	*Leucanthemum vulgare* Lam.
Asterales	ASTERACEAE	*Scorzoneroides autumnalis* (L.) Moench
Asterales	ASTERACEAE	*Taraxacum officinale* aggr.
Brassicales	BRASSICACEAE	*Cardamine prantesis* L.
Caryophyllales	CARYOPHYLLACEAE	*Cerastium fontanum* Baumg.
Caryophyllales	POLYGONACEAE	*Rumex acetosa* L.
Fabales	FABACEAE	*Lathyrus pratensis L.*
Fabales	FABACEAE	*Lotus corniculatus* L.
Fabales	FABACEAE	*Trifolium pratense* L.
Fabales	FABACEAE	*Trifolium repens* L.
Fabales	FABACEAE	*Vicia sepium L.*
Lamiales	LAMIACEAE	*Prunella vulgaris* L.
Lamiales	OROBANCHACEAE	*Euphrasia* agg.
Lamiales	OROBANCHACEAE	*Rhinanthus minor* L.
Lamiales	PLANTAGINACEAE	*Plantago lanceolata* L.
Lamiales	PLANTAGINACEAE	*Veronica chamaedrys* L.
Lamiales	PLANTAGINACEAE	*Veronica serpyllifolia L.*
Poales	POACEAE	*Agrostis capillaris* L.
Poales	POACEAE	*Agrostis stolonifera* L.
Poales	POACEAE	*Anthoxanthum odoratum* L.
Poales	POACEAE	*Festuca rubra* L.
Poales	POACEAE	Grass
Poales	POACEAE	*Holcus lanatus* L.
Ranunculales	RANUNCULACEAE	*Caltha palustris* L.
Ranunculales	RANUNCULACEAE	*Ranunculus acris* L.
Ranunculales	RANUNCULACEAE	*Ranunculus bulbosus* L.
Ranunculales	RANUNCULACEAE	*Ranunculus repens* L.

Table 2. List of species of flower visitors

Order	Family	Specie
Coleoptera	DASCILLIDAE	*Dascillus ceruinus* (Linnaeus, 1758)
Coleoptera	ELATERIDAE	*Ctenicera cuprea* (Fabricius, 1775)
Coleoptera	NITIDULIDAE	*Meligethes* aff. *aeneus* (Fabricius, 1775)
Diptera	AGROMYZIDAE	*Phytomyza* aff. *fallaciosa* Brischke, 1880
Diptera	ANTHOMYIIDAE	*Botanophila brunneilinea* (Zetterstedt, 1845)
Diptera	ANTHOMYIIDAE	*Botanophila* sp.
Diptera	ANTHOMYIIDAE	*Botanophila striolata* (Fallén, 1824)
Diptera	ANTHOMYIIDAE	*Delia platura* (Meigen, 1826)
Diptera	ANTHOMYIIDAE	*Delia* sp.
Diptera	ANTHOMYIIDAE	*Hylemya variata* (Fallén, 1823)
Diptera	ANTHOMYIIDAE	*Pegoplata aestiva* (Meigen, 1826)
Diptera	ANTHOMYIIDAE	*Pegoplata infirma* (Meigen, 1826)
Diptera	BIBIONIDAE	*Dilophus femoratus* Meigen, 1804
Diptera	CALLIPHORIDAE	*Calliphora vomitoria* (Linnaeus, 1758)
Diptera	CALLIPHORIDAE	*Lucilia caesar* (Linnaeus, 1758)
Diptera	CALLIPHORIDAE	*Melanomya nana* (Meigen, 1826)
Diptera	CERATOPOGONIDAE	Ceratopogonidae sp.
Diptera	CERATOPOGONIDAE	*Mallochohelea* sp.
Diptera	CHAMAEMYIIDAE	*Chamaemyia herbarum* (Robineau-Desvoidy, 1830)
Diptera	CHLOROPIDAE	*Chlorops hypostigma* Meigen, 1830
Diptera	CHLOROPIDAE	*Chlorops meigenii* Loew, 1866
Diptera	CHLOROPIDAE	*Chlorops serenus* Loew, 1866
Diptera	DOLICHOPODIDAE	*Chrysotus* sp.
Diptera	DOLICHOPODIDAE	*Dolichopus plumipes* (Scopoli, 1763)
Diptera	DOLICHOPODIDAE	*Thyripticus* sp.
Diptera	DROSOPHILIDAE	*Scaptomyza flava* (Fallén, 1823)
Diptera	EMPIDIDAE	*Empis digramma* Meigen in Gistl, 1835
Diptera	EMPIDIDAE	*Empis femorata* Fabricius, 1798
Diptera	EMPIDIDAE	*Empis livida* Linnaeus, 1758
Diptera	EMPIDIDAE	*Empis punctata* Meigen, 1804
Diptera	EMPIDIDAE	*Hilara* sp.
Diptera	EPHYDRIDAE	*Hydrellia maura* Meigen, 1838
Diptera	EPHYDRIDAE	*Hydrellia* sp.
Diptera	FANNIIDAE	*Fannia rondanii* (Strobl, 1893)
Diptera	FANNIIDAE	*Fannia* sp.
Diptera	FANNIIDAE	*Fannia umbratica* Collin, 1939
Diptera	HYBOTIDAE	Bicellaria sp.
Diptera	HYBOTIDAE	*Bicellaria sulcata* (Zetterstedt 1842)
Diptera	HYBOTIDAE	*Hybos femoratus* (Müller, 1776)
Diptera	MUSCIDAE	*Coenosia pedella* (Fallén, 1825)
Diptera	MUSCIDAE	*Drymeia brumalis* (Rondani, 1866)
Diptera	MUSCIDAE	*Drymeia hastata* (Harris, [1780])
Diptera	MUSCIDAE	*Helina* sp.
Diptera	MUSCIDAE	*Helina subvittata* (Séguy, 1923)
Diptera	MUSCIDAE	*Hydrotaea irritans* (Fallén, 1823)

Diptera	MUSCIDAE	*Myospila meditabunda* (Fabricius, 1781)
Diptera	MUSCIDAE	*Neomyia cornicina* (Fabricius, 1781)
Diptera	MUSCIDAE	*Phaonia incana* (Wiedemann, 1817)
Diptera	MUSCIDAE	*Phaonia serva* (Meigen, 1826)
Diptera	MUSCIDAE	*Phaonia* sp.
Diptera	MUSCIDAE	*Thricops longipes* (Zetterstedt, 1845)
Diptera	MUSCIDAE	*Thricops nigritellus* (Zetterstedt, [1838])
Diptera	MUSCIDAE	Thricops *sp.*
Diptera	NA	Diptera sp.
Diptera	OPOMYZIDAE	*Geomyza tripunctata* Fallén, 1823
Diptera	OPOMYZIDAE	*Opomyza germinationis* (Linnaeus, 1758)
Diptera	OPOMYZIDAE	*Opomyza petrei* Mesnil, 1934
Diptera	RHAGIONIDAE	*Stymphoromyia crassicornis* (Panzer, [1806])
Diptera	SCATHOPHAGIDAE	*Scathophaga stercoraria* (Linnaeus, 1758)
Diptera	SCATHOPHAGIDAE	Scathophagidae sp.
Diptera	SEPSIDAE	*Themira lucida* (Staeger in Schiødte, 1844)
Diptera	SPHAEROCERIDAE	*Lotophila atra* (Meigen, 1830)
Diptera	SYRPHIDAE	*Cheilosia albitarsis/ranunculi*
Diptera	SYRPHIDAE	*Cheilosia antiqua* (Meigen, 1822)
Diptera	SYRPHIDAE	*Chrysogaster virescens* Loew, 1854
Diptera	SYRPHIDAE	*Eristalis horticola* (De Geer, 1776)
Diptera	SYRPHIDAE	*Eristalis tenax* (Linnaeus, 1758)
Diptera	SYRPHIDAE	*Eupeodes corollae* (Fabricius, 1794)
Diptera	SYRPHIDAE	*Eupeodes* sp.
Diptera	SYRPHIDAE	*Lejogaster metallina* (Fabricius, 1781)
Diptera	SYRPHIDAE	*Melanostoma mellinum* (Linnaeus, 1758)
Diptera	SYRPHIDAE	*Platycheirus albimanus* (Fabricius, 1781)
Diptera	SYRPHIDAE	*Platycheirus clypeatus* (Meigen, 1822)
Diptera	SYRPHIDAE	*Platycheirus manicatus* (Meigen, 1822)
Diptera	SYRPHIDAE	*Sphaerophori*a sp.
Diptera	SYRPHIDAE	*Syritta pipiens* (Linnaeus, 1758)
Diptera	SYRPHIDAE	*Syrphus vitripennis* Meigen, 1822
Diptera	SYRPHIDAE	*Volucella bombylans* (Linnaeus, 1758)
Diptera	TABANIDAE	*Haematopota pluvialis* (Linnaeus, 1758)
Diptera	TACHINIDAE	Tachinidae sp.
Diptera	THEREVIDAE	*Dialineura anilis* (Linnaeus, 1761)
Heteroptera	CICADELLIDAE	Cicadellidae sp.
Heteroptera	MIRIDAE	Miridae sp.
Hymenoptera	APIDAE	*Bombus hortorum* (Linnaeus, 1761)
Hymenoptera	APIDAE	*Bombus lucorum* (Linnaeus, 1761)
Hymenoptera	APIDAE	*Bombus pascuorum* (Scopoli, 1763)
Hymenoptera	APIDAE	*Bombus pratorum* (Linnaeus, 1761)
Hymenoptera	APIDAE	*Bombus terrestris* (Linnaeus, 1758)
Hymenoptera	ICHNEUMONIDAE	Ichneumonidae sp.1
Hymenoptera	TENTHREDINIDAE	*Tenthredo arcuata* Foster, 1771
Lepidoptera	CRAMBRIDAE	*Agriphila tristella* ([Denis & Schiffermuller], 1775
Lepidoptera	NA	Lepidoptera sp.
Lepidoptera	PSYCHIDAE	*Psyche casta* (Pallas, 1767)

Table 3. List of species of leaf miners

Order	Family	Specie
Diptera	AGROMYZIDAE	*Agromyza nana* Meigen, 1830
Diptera	AGROMYZIDAE	*Agromyza nigripes* Meigen, 1830
Diptera	AGROMYZIDAE	Agromyzae sp.1
Diptera	AGROMYZIDAE	Agromyzidae grass sp.1
Diptera	AGROMYZIDAE	Agromyzidae grass sp.2
Diptera	AGROMYZIDAE	Agromyzidae grass sp.3
Diptera	AGROMYZIDAE	Agromyzidae grass sp.4
Diptera	AGROMYZIDAE	Agromyzidae grass sp.5
Diptera	AGROMYZIDAE	*Cerodonta denticornis* (Panzer, [1806])
Diptera	AGROMYZIDAE	*Cerodonta muscina* (Meigen, 1830)
Diptera	AGROMYZIDAE	*Cerodontha fulvipes* (Meigen, 1830)
Diptera	AGROMYZIDAE	*Chromatomya horticula/syngenesiae*
Diptera	AGROMYZIDAE	*Chromatomyia milii* (Kaltenbach, 1864)
Diptera	AGROMYZIDAE	*Chromatomyia nigra* (Meigen, 1830)
Diptera	AGROMYZIDAE	*Chromatomyia paracillata* Godfray, 1985
Diptera	AGROMYZIDAE	*Liriomyza taraxaci* Hering, 1927
Diptera	AGROMYZIDAE	*Lyriomyza flaveola* (Fallén, 1823a)
Diptera	AGROMYZIDAE	*Phytomyza crassiseta* Zetterstedt, 1860
Diptera	AGROMYZIDAE	*Phytomyza fallaciosa* Brischke, 1880
Diptera	AGROMYZIDAE	*Phytomyza leucanthemi* Hering, 1935
Diptera	AGROMYZIDAE	*Phytomyza notata* Meigen, 1830
Diptera	AGROMYZIDAE	*Phytomyza plantaginis* Goureau, 1851
Diptera	AGROMYZIDAE	*Phytomyza ranunculi* (Schrank, 1803)
Diptera	AGROMYZIDAE	*Phytomyza ranunculivora* Hering, 1932
Diptera	AGROMYZIDAE	*Phytomyza stolonifera* Hering, 1949
Diptera	ANTHOMYIIDAE	*Pegomyia solennis* (Meigen, 1826)
Diptera	CHLOROPIDAE	*Chlorops hypostigma* Meigen, 1830
Diptera	DROSOPHILIDAE	*Scaptomyza flava* (Fallén, 1823)
Diptera	DROSOPHILIDAE	*Scaptomyza graminum* (Fallén, 1823)
Diptera	EPHYDRIDAE	*Hydrellia maura* Meigen, 1838
Lepidoptera	ELASCHISTIDAE	*Elachista* sp.
Lepidoptera	GRACILLARIOIDEA	*Bucculatrix nigricomella* (Zeller, 1839)

Table 4. List of species of parasitoids.

Order	Family	Specie
Hymenoptera	BRACONIDAE	*Apodesmia irregularis* (Wesmael, 1835)
Hymenoptera	BRACONIDAE	*Apodesmia similis* (Szépligeti, 1898)
Hymenoptera	BRACONIDAE	*Chorebus aphantus* (Marshall, 1896)
Hymenoptera	BRACONIDAE	*Chorebus asramenes* (Nixon, 1945)
Hymenoptera	BRACONIDAE	*Chorebus conjugens* (Nees von Esenbeck, 1812)
Hymenoptera	BRACONIDAE	*Chorebus diremtus* (Nees, 1834)
Hymenoptera	BRACONIDAE	*Chorebus longicornis* (Nees, 1811)
Hymenoptera	BRACONIDAE	*Dacnusa areolaris* (Nees, 1811)
Hymenoptera	BRACONIDAE	*Dacnusa faeroeensis (Roman, 1917)*
Hymenoptera	BRACONIDAE	*Dacnusa hospita* (Förster, 1862)
Hymenoptera	BRACONIDAE	*Dacnusa laevipectis* Thomson, 1895
Hymenoptera	BRACONIDAE	*Dacnusa macrospila* (Haliday, 1839)
Hymenoptera	BRACONIDAE	*Dacnusa maculipes* Thomson, 1895
Hymenoptera	BRACONIDAE	*Dacnusa plantaginis* Griffiths, 1967
Hymenoptera	BRACONIDAE	*Exotela gilvipes* (Haliday, 1839)
Hymenoptera	BRACONIDAE	*Grammospila rufiventris* (Nees, 1812)
Hymenoptera	BRACONIDAE	*Opius* sp.BOLD:ACR0288
Hymenoptera	BRACONIDAE	*Opius* sp.BOLD:ADH4705
Hymenoptera	BRACONIDAE	*Phaedrotoma caesa* (Haliday, 1837)
Hymenoptera	BRACONIDAE	*Phaedrotoma* sp.BOLD:ADF0710
Hymenoptera	EULOPHIDAE	*Chrysocharis pallipes* (Nees, 1834)
Hymenoptera	EULOPHIDAE	*Chrysocharis viridis* (Nees, 1834)
Hymenoptera	EULOPHIDAE	*Diglyphus isaea* (Walker)
Hymenoptera	EULOPHIDAE	*Diglyphus* sp.BOLD:ADI0604
Hymenoptera	EULOPHIDAE	*Hemiptarsenus fulvicollis* Westwood, 1833
Hymenoptera	EULOPHIDAE	*Hemiptarsenus unguicellus* Westwood, 1833
Hymenoptera	EULOPHIDAE	*Hemiptsarsenus ornatus* (Nees, 1834)
Hymenoptera	EULOPHIDAE	*Pnigalio* sp.BOLD:ADI0573
Hymenoptera	NA	Chalcidoidea sp.
Hymenoptera	NA	Chalcidoidea sp.2
Hymenoptera	PLATYGASTRIDAE	Platygastridae sp.BOLD:ADH7962
Hymenoptera	PTEROMALIDAE	*Microgaster maculata* Walker, 1833